Epidemic and Pandemic Influenza in Tropical Singapore – Impact and Effectiveness of Response Strategies

A Thesis submitted for the degree of Doctor of Philosophy

of The Australian National University

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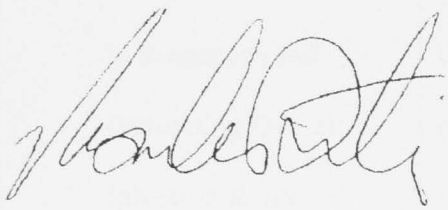
Statement of Work

This thesis and the publications within were performed by me while enrolled at the Australian National University. The thesis is entirely my own original work, which was taken from my own research in collaboration with the authors listed in the individual publications.

The contributions made by me in the individual publications presented in the thesis are described in detail in Chapter One.



Dr Vernon Lee

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Table of Contents

	Semi-Closed Environments	among young adult military personnel with febrile respiratory illness in Singapore. PLoS One. 2011 Mar 2;6(3):e17468.	
7	Preparedness and Response to Influenza Epidemics and Pandemics	Lee VJ, Lye DC, Wilder-Smith A. Combination strategies for pandemic influenza response - a systematic review of mathematical modeling studies. BMC Medicine. 2009;7:76.	59 – 76
8	Influenza Vaccine Cross-Reactivity	Lee VJ, Tay JK, Chen MIC, et al. Inactivated trivalent seasonal influenza vaccine induces limited cross-reactive neutralizing antibody responses against 2009 pandemic and 1934 PR8 H1N1 strains. Vaccine. 2010 Oct 4;28(42):6852-7.	77 – 80
9	Effectiveness of Oseltamivir Ring Prophylaxis	Lee VJ, Yap J, Cook AR, et al. Oseltamivir ring prophylaxis for containment of Influenza A (H1N1-2009) outbreaks. New England Journal of Medicine. 2010;362:2166-74.	81 – 84
10	Seroconversion and Asymptomatic Infections during	Lee VJ, Yap J, Tay JK, et al. Seroconversion and asymptomatic infections during oseltamivir prophylaxis	85 – 88

List of Tables

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ABSTRACT

Background

Influenza is a disease of global significance, including in tropical regions where it spreads throughout the year. Understanding the spread and impact of influenza in the tropics is therefore important for preparedness planning. While there are numerous pharmaceutical and public health measures that attempt to reduce the spread and impact of influenza, few conclusive epidemiological studies are available to document their effectiveness. Scientific evidence is especially lacking for pandemic preparedness and response measures due to the rarity of pandemics. Singapore, a globally-connected, tropical Asian city-state, provides an excellent platform to determine the spread and impact of influenza in the tropics, and the effectiveness of public health measures in reducing the impact.

Aims

This thesis aims to detail the impact of epidemic and pandemic influenza in Singapore, and to assess the effectiveness of various assessment and response measures in Singapore during the 2009 H1N1 influenza pandemic.

Results

Influenza epidemics and pandemics were the likely cause of most excess mortality periods in Singapore from 1950 to 2000. Good surveillance is therefore important to detect epidemics for appropriate response. During the 2009 H1N1 influenza epidemic in Singapore, different methods for estimating influenza infection rates provided comparable findings if accurate input parameters were used. There are advantages and

disadvantages to each method, and multiple methods should be used where possible for cross validation. One such method, a seroepidemiology cohort study, showed a 13% seroconversion rate in adults in the community and lower rates among hospital workers, suggesting that most of the population remained susceptible and required further protection. A surveillance program in the Singapore military during the peri-pandemic period showed the different clinical presentation of influenza compared to non-influenza cases, and introduced a clinical diagnostic model to help predict influenza among febrile respiratory illness cases for management.

The possible effectiveness of combination strategies in reducing the impact of influenza was shown via a systematic review of mathematical modeling studies. It provides new evidence for the effectiveness of different strategies to reduce the spread of influenza in the military setting. One study showed that influenza vaccination may confer cross protection to other H1N1 strains, and previous exposure to pre-1957 H1N1 strains may confer some protection against the 2009 H1N1 strain. Another study showed the effectiveness of post-exposure ring prophylaxis with oseltamivir, together with prompt outbreak detection and isolation, as a containment strategy to reduce influenza spread. In the same setting, cessation of post-exposure prophylaxis did not result in subsequent disproportionate increase in infection rates, and asymptomatic infections occurred which may confer additional protection against future infection. While prophylaxis failures occurred, none were due to mutations that conferred resistance. Another study documented that public health measures such as enhanced surveillance with isolation, segregation and social distancing, and wearing personal protective equipment limited transmission of influenza.

Conclusions

This thesis provides substantial contribution to the existing knowledge on influenza. It is important for public health professionals and decision makers to learn from the findings, and to use them as a platform for policy making.

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Acknowledgements

I dedicate this PhD thesis to my wife, Jocelyn, who has steadfastly stood by me throughout my medical post-graduate training, and has been a constant source of encouragement and support through my endeavors including this PhD.

I thank my PhD supervisors, Associate Professor Paul Kelly and Associate Professor Kamalini Lokuge from the Australian National University, and Professor Chia Kee Seng from the National University of Singapore for their invaluable guidance in the research and development of the thesis. I also thank all of my collaborators who have worked with me on the studies which have been included in this thesis – their names are shown in the co-authorship and acknowledgement sections in the individual manuscripts. This displays the importance of close collaboration among researchers, and the success it brings. I also thank the staff of the Singapore Armed Forces Biodefence Center for their unwavering support and teamwork as we came face-to-face with and successfully mitigated the impact of the 2009 H1N1 influenza pandemic in Singapore.

I thank my superiors in the Singapore Armed Forces, BG (DR) Benjamin Seet and COL (DR) Kang Wee Lee, for their support and encouragement as I undertook this PhD. I also thank my long time mentors, Professor Goh Kee Tai and Professor Lee Hin Peng for guiding me through my formative years and enabling me to be where I am today in my career and education.

I thank Ms Barbara Bowen from the Australian National University for assisting with the administrative processes through the PhD; Ms Fiona MacIver for assisting with

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I thank all of my medical and public health colleagues and friends globally whom I have met in the course of my work. They have provided the inspiration for me to embark on my journey in public health. Finally, I thank my family and friends for their moral support.

I also acknowledge the respective journals that have kindly allowed me to use the original journal publications for this thesis. The copyright permission, where applicable, is indicated in the credit line at the end of the respective chapter where the publication is presented.

Glossary of Acronyms

GMT	Geometric mean titers (the arithmetic mean of the logarithmic titers obtained from the laboratory test)
HA	Haemagglutinin (a surface protein on the influenza virus)
HAI	Haemagglutination inhibition (ability of antibodies to prevent the agglutination of red blood cells by virus antigens by forming antibody-antigen complexes)
ICMJE	International Committee of Medical Journal Editors
ILI	Influenza-like illness (group of illnesses that have fever, and respiratory symptoms such as cough and sore throat)
LR	Likelihood ratio (likelihood of a result occurring in one group compared to the likelihood of the result in another group)
MDCK	Madin-Darby Canine Kidney (cell lines grown from the kidney tissue of an adult canine)
NA	Neuraminidase (a surface protein on the influenza virus)
NPV	Negative predictive value (proportion of test subjects with a negative test result who are correctly identified as negative)
PCR	Reverse transcription polymerase-chain reaction
PPV	Positive predictive value (proportion of test subjects with a positive test result who are correctly identified as positive)
R_0	Basic reproductive number (the average number of secondary cases produced by a primary case)
RNA	Ribonucleic acid
SARS	Severe acute respiratory syndrome
WHO	World Health Organization

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Chapter One

Introduction

Influenza is a respiratory disease that causes annual seasonal epidemics with substantial disease burden globally. In addition, once in every few decades pandemics occur which result in devastating health and socio-economic consequences globally. A substantial proportion of the world's population is affected each year by seasonal epidemic influenza, and this proportion may increase with pandemic influenza. Due to the propensity for the influenza virus to mutate, populations are constantly at risk of seasonal influenza epidemics, and are almost completely susceptible to novel pandemic strains as they emerge. The current global socio-cultural landscape promotes the close interaction between humans, birds which are the natural hosts of all known influenza subtypes, and animals which can harbor both avian and human strains of influenza. This results in the unique opportunity for the re-assortment and emergence of new influenza strains which threaten to spread across the world.

Influenza is well recognized as a disease of significance in temperate regions due to the clear seasonal peaks in winter. In temperate countries, seasonal influenza epidemics occur during the winter season with influenza activity increasing high above baseline levels for six to eight weeks as a single peak, while baseline influenza activity remains relatively low throughout the rest of the year (1). Influenza in tropical regions, on the other hand, lacks well-defined seasons and spreads throughout the year with a high baseline incidence and frequently more than one epidemic peak annually (2). Studies in sub-tropical Hong Kong and tropical Singapore have shown that the mortality and morbidity due to influenza is of similar magnitude compared to temperate countries (3-6). It is therefore important for public health practitioners and policy makers to understand how influenza epidemics and pandemics spread in tropical regions.

In addition, there are numerous pharmaceutical and public health measures that have been used in an attempt to reduce the spread and impact of influenza. While there has been evidence of the effectiveness of some of these measures, sufficient direct scientific evidence is lacking for most, especially for proposed pandemic preparedness and response measures, due to the relative rarity of influenza pandemics. There is therefore an urgent and important need to determine the effectiveness of these measures where the opportunity arises, and to contribute to an improved evidence base.

Singapore, a globally-connected, tropical Asian city-state located at the equator, is well poised to perform the necessary surveillance and research to detail the spread and impact of influenza in the tropical setting. In addition, Singapore was affected substantially by the 2009 influenza H1N1 pandemic, which provides an excellent platform to determine the extent to which public health measures influence the impact of influenza in the local setting.

Aims and Overview of the Thesis

The aims of this thesis are:

1) to detail the impact of epidemic and pandemic influenza in Singapore, providing additional evidence on the impact of influenza in tropical regions; and
2) to assess the effectiveness of various assessment and response measures using data obtained in Singapore during the spread of 2009 pandemic influenza.

This will be important for preparedness and response planning for future pandemics of influenza and other similar respiratory diseases.

The thesis is based on a series of ten published journal manuscripts, organized around individual chapters, that collectively address the aims by presenting new evidence on different components of the impact, assessment, and response during epidemic and pandemic influenza. In addition, there is a background chapter which provides the context for the published work, and a concluding chapter which ties the evidence together and suggests important research questions for the future. At the start of each chapter, there is a short background section to provide a link to the previous chapter, as well as to introduce the next manuscript.

The second chapter of the thesis introduces aspects of influenza viruses which are important for the understanding of the disease. It also describes the development of epidemics and pandemics, and lays the foundation for the rest of the thesis.

The third chapter focuses on influenza epidemics and pandemics in the tropics, and includes publications on the excess mortality due to influenza in Singapore from 1950 to 2000; and also the spread and impact of the three 20th Century pandemics in Singapore. This provides the backdrop for the importance of influenza prevention in tropical countries such as Singapore.

The fourth chapter introduces the reader to the 2009 H1N1 influenza pandemic in Singapore. It then uses the 2009 pandemic as a backdrop to present a methodological

paper on the comparability between different methods for estimating influenza infection rates, and the importance of all of these methods in different settings.

The fifth chapter continues from the fourth chapter by providing a detailed analysis of the specific seroepidemiology cohort study to determine infection rates among different cohorts during the first epidemic wave of 2009 H1N1 pandemic influenza in Singapore.

The sixth chapter introduces the unique differences in the spread of influenza in closed and semi-closed environment such as military institutions and schools, and the importance of surveillance in such environments. It then showcases one such surveillance program in the Singapore military to determine the epidemiology and clinical features of influenza in a semi-closed military setting during the time of the 2009 influenza pandemic. This surveillance program is also important to show the differences in clinical presentation of influenza compared to non-influenza cases, and among different influenza strains. Pursuant to the previous chapter, it also illustrates the difficulty of using influenza-like illness (ILI) or other clinical criteria for diagnosis without accompanying laboratory testing.

The seventh chapter provides a detailed overview of the importance of preparedness and response strategies to reduce the spread and impact of influenza, and the current evidence for these strategies. It starts off with a systematic review of mathematical modeling studies which show the effectiveness of combination strategies in reducing the impact of influenza.

The eighth to twelfth chapters then move into new evidence for the effectiveness of different strategies for influenza prevention and response in a semi-closed military environment. This includes the cross-reactivity of influenza vaccines to other non-vaccine strains, the effectiveness of post-exposure ring prophylaxis with oseltamivir as a containment strategy, and the effectiveness of various public health measures in reducing the spread of influenza.

The thirteenth and final chapter discusses the impact of these studies in providing additional evidence and understanding of influenza in the tropics, and the effectiveness of different measures to prevent its spread, especially in closed and semi-closed environments where influenza can have substantial impact. It suggests gaps for additional research, and proposes a framework for future studies to address these gaps and improve the evidence-base to deal with the threat of influenza in the future.

This research provides a substantial contribution to the body of knowledge on influenza and its prevention. It will be important for public health professionals and decision makers worldwide to learn from the lessons described herewith, and to use this as a platform for policy making.

Contributions to the Manuscripts

In all except one of the manuscripts presented in this thesis, I was the lead author and main contributor to the entire process from study conception to publication. For one paper (7), I was the second author with the most substantial contribution after the lead author.

To provide a representation of my contributions to each manuscript, I have presented in Table 1 the estimated contribution to the different aspects of the study based on the criteria for authorship by the International Committee of Medical Journal Editors (ICMJE) uniform requirements for manuscripts submitted to biomedical journals (available at http://www.icmje.org/ethical_1author.html). From the ICMJE criteria, I have assessed my contributions to the studies as percentages in each of the following four areas: 1) conception and design; 2) acquisition of data; 3) analysis and interpretation of data; 4) drafting the article. The approval for publication is given for all manuscripts.

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Table 1. Estimate of my contributions (in percentages) to different areas of each study included as manuscripts in the thesis chapters

Chapter	Manuscript Title	Journal	Manuscript Type	Number of co-authors	Conception and Design (%)	Acquisition of data (%)	Analysis and interpretation of data (%)	Drafting the article (%)
3	Influenza excess mortality from 1950-2000 in tropical Singapore	PLoS One	Original research	8	65	70	65	70
4	Comparability of different methods for estimating influenza infection rates over a single epidemic wave	American Journal of Epidemiology (AJE)	Original research	12	65	55	60	75
5	2009 Influenza H1N1 seroconversion rates and risk factors among distinct adult cohorts in Singapore	Journal of the American Medical Association	Original research	18	35	30	30	30

		(JAMA)						
6	A clinical diagnostic model for predicting influenza among young adult military personnel with febrile respiratory illness in Singapore.	PLoS One	Original research	17	80	75	70	80
7	Combination strategies for pandemic influenza response - a systematic review of mathematical modeling studies.	BMC Medicine	Systematic review	2	80	85	80	80
8	Inactivated trivalent seasonal influenza vaccine induces limited cross-reactive neutralizing antibody responses against 2009	Vaccine	Original research	15	65	65	60	65

	pandemic and 1934 PR8 H1N1 strains.							
9	Oseltamivir ring prophylaxis for containment of Influenza A (H1N1-2009) outbreaks.	New England Journal of Medicine (NEJM)	Original research	17	70	80	60	70
10	Seroconversion and asymptomatic infections during oseltamivir prophylaxis against Influenza A H1N1 2009.	BMC Infectious Diseases	Original research	10	80	80	70	80
11	Investigation of Causes of Oseltamivir Chemoprophylaxis Failures during Influenza A (H1N1-2009) outbreaks.	Journal of Clinical Virology (JCV)	Original research	12	70	60	55	70

12	Effectiveness of public health measures in mitigating pandemic influenza spread: A prospective serological cohort study.	Journal of Infectious Diseases (JID)	Original research	16	80	80	65	70

Chapter Two

Influenza Viruses

Influenza viruses are single-stranded ribonucleic acid (RNA) viruses belonging to the *Orthomyxoviridae* family. There are three types of influenza viruses – influenza A, B, and C, differentiated by their nucleus, matrix, and surface proteins which have different antigenic properties. This thesis focuses on influenza A viruses which commonly circulate among humans, birds, and other mammals such as pigs, and has the propensity for causing human epidemics and pandemics. The thesis also occasionally refers to influenza B viruses that are in current circulation globally and are detected through epidemiological surveillance as they are also a common cause of human epidemics.

Influenza A viruses are categorized into subtypes by two different surface proteins, the haemagglutinin (HA) and neuraminidase (NA) proteins. These subtypes are further categorized into strains which differ by specific genetic sequences. The HA protein facilitates the binding of the influenza virus to the host cell through sialic acid receptors, enabling entry into and infection of the host cell for replication. Once the replication of viral progeny is completed inside the host cell, the NA protein cleaves the sialic acid receptors to allow the progeny to be released from the infected cell, enabling further infection of other cells. There are currently sixteen known haemagglutinin proteins types and nine neuraminidase protein types and all influenza subtypes can be found in aquatic birds (1,2). In humans, only H1N1, H2N2, and H3N2 subtypes have been documented to be in widespread circulation in humans in the past century with efficient transmission from person to person. Other mammals have been known to be infected with the influenza virus, and pigs are common alternate hosts for influenza viruses and can harbor both avian and human viruses. Pigs are therefore an important platform in the reassortment and emergence of new

viral strains - this was the hypothesized mechanism leading up to the influenza pandemic in 2009 among humans (1,3).

Spread and Clinical Features of Influenza

Influenza spreads by droplet, contact and airborne modes, but the relative likelihood of spread through each of these modes varies. The main mode is believed to be through droplets (aqueous particles >10μm containing virus particles) which are formed by actions such as coughing and sneezing (4,5). Influenza can also spread through airborne aerosols of influenza particles (<5μm) that are expelled also through coughing and sneezing, or from medical procedures such as nebulisation or intubation (4-6)- although aerosol transmission is believed to be a less common mode of transmission (7). Finally, contact with infected material such as contaminated fomites which are then transferred to the respiratory tract (directly or indirectly through hands) are also thought to result in substantial transmission (8,9).

The incubation period of influenza is about one to four days (10). Detection of influenza viruses in respiratory samples have been shown to occur one to two days before the onset of symptoms until about five days after; and detection can occur for two weeks or more after the onset of illness in children and immunocompromised individuals (11-16). For the novel 2009 H1N1 pandemic influenza virus, viral shedding may last longer with one study having cultured viruses in about a quarter of samples taken seven days after the onset of illness (17). The likely infectious period for influenza is thought of to coincide with viral detection in respiratory samples, but it is not known how virus detection in respiratory samples correlates with infectiousness and intensity of spread. Infection may also be sub-clinical without

significant clinical symptoms, and these cases are difficult to detect while still likely to be able to transmit the virus (18).

Clinical attack rates during seasonal influenza epidemics range from 10% to 20%, and may be higher in pandemics depending on population susceptibility to the circulating influenza strain (19). The estimated basic reproductive number (R_0, the average number of secondary cases produced by a primary case) ranges from less than two for seasonal epidemics in the general population, to less than four for the 1918 pandemic (20,21). This is overall much lower than other infectious diseases with high transmissibility such as measles or varicella. However, as described in later chapters, the R_0 and overall spread of influenza varies substantially from sub-population to sub-population, with some groups being at high risk of increased spread due to various reasons discussed later.

Influenza causes a wide range of clinical symptoms including: fever; cough; sore throat; myalgia; rhinorrhea; nasal congestion; weakness; loss of appetite; headache; and gastro-intestinal symptoms. Fever usually resolves after five days but myalgia and other symptoms often last for up to and beyond two weeks. Respiratory symptoms are the result of local cellular damage and apoptosis due to viral infection, together with inflammation; while host immune responses produce cytokines and other immune modulators that result in fever and other systemic presentations such as headache, myalgia, anorexia, and malaise (16,22). Occasionally, severe inflammation may result in primary viral pneumonia, and the loss of epithelial and ciliated cells in the respiratory tract increases the susceptibility of the host to secondary bacterial infections. There are unfortunately no unique signs or symptoms that describe

influenza illness, and influenza cases are difficult to differentiate from other similar illnesses caused by a variety of other viruses and bacteria; these are therefore collectively called influenza-like illnesses (ILIs). The WHO definition of ILI is sudden onset fever of more than 38.0°C and cough or sore throat in the absence of other diagnoses (23).

There have been several studies exploring the clinical presentation that predicts influenza infection but the results have been mixed. The accuracy of the set of symptoms and signs in predicting influenza infection is highly dependent on the local context, especially the other circulating ILIs and the demographics of the local population. One systematic review using summary statistics from several selected studies found that there were no overall symptoms or signs that had a summary likelihood ratio for influenza of greater than two (24). Only the absence of fever (Likelihood Ratio (LR) 0.40), cough (LR 0.42), and nasal congestion (LR 0.49) had likelihood ratios of less than 0.5, showing a decreased likelihood of influenza if these symptoms were absent (24). The ILI definition was found in a recent study to only have sensitivity of 73.8%, specificity 43.0%, positive predictive value (PPV) 39.5% and negative predictive value (NPV) 76.5% in identifying influenza cases (25). It is therefore useful to consider the difficulty in defining the set of symptoms and signs that exactly describe influenza infection, and that other modalities for surveillance are necessary. These will be discussed further in Chapter Six.

Laboratory Testing for Influenza Viruses

Laboratory testing provides a more definitive method of determining infection from influenza viruses, especially to identify the unique influenza strain. This is important

in the context of understanding the epidemiology of influenza and the effectiveness of various interventions in preventing the spread of the disease. At the same time, laboratory testing requires additional resources and depending on the test method, may pose a substantial challenge in lower resource settings, especially at point-of-care clinical consultations. This section describes some of the common laboratory tests for influenza – these form the foundation to identify influenza infection for the studies presented in this thesis.

Virus culture is usually considered the gold standard for influenza diagnosis. Cultures are performed on clinical samples which include throat or nasopharyngeal swabs, to nasopharyngeal or bronchial washes or aspirates. It can only yield positive cultures if there is live virus in the clinical samples, and therefore false negative tests often occur. Standard viral cultures in Madin-Darby Canine Kidney (MDCK) cells or inoculation of eggs take about three to 10 days to yield sufficient virus for identification, limiting its immediate use in clinical and public health settings (26). Rapid cell cultures (for example shell cultures or cell mixtures) can reduce test times to about one to three days, although this is still far longer than the other tests described below. The virus obtained from the viral cultures will still have to be tested to determine the subtype or strain, using another laboratory method such as reverse transcription polymerase-chain reaction (PCR) haemagglutination inhibition (HAI) tests, or immunofluoresence tests mentioned below. Due to the substantial quantities of virus produced that can be used for further tests, virus cultures are often the foundation for further genetic sequencing studies that can determine the specific influenza strain and the presence of any genetic differences such as mutations.

PCR is a popular testing method and is widely used to identify influenza strains if appropriate reagents are available to detect strain-specific regions of the viral genome. PCR tests need to be performed in specialized laboratories by trained technicians, and are able to yield results within four to six hours which makes them useful for clinical and public health interventions. In addition, PCR is able to detect viral genetic material as long as it is part of the detection region of the reagent, regardless of whether the genetic material is only a virus fragment or a whole live virus. It is therefore very sensitive for influenza infection, but a positive test may not necessarily correlate with infectiousness as sufficient live virus may not be present.

Antibody tests are also used for epidemiological studies due to their ability to determine prior infection in the absence of respiratory samples. They use blood samples and detect the antibodies against the influenza strain being tested. The two most commonly used tests in research studies are neutralization tests or HAI. Influenza neutralization tests are based on the inhibition of the influenza virus's pathogenic effect on MDCK cells by the subject's serum at different dilutions or titers. This is often a very resource and time intensive process, and current testing frequently uses microneutralization tests in which staining dyes are used to detect cells that have been infected by the influenza virus. Although microneutralization tests are less resource intensive compared to traditional neutralization assays, they are still more resource intensive when compared to other laboratory methods. An HAI test, on the other hand, uses the property of the influenza virus to agglutinate red blood cells, and can be performed relatively quickly with results in less than one hour (excluding sample preparation time). Red blood cells often used for testing for human influenza viruses include turkey, guinea pig, and human type O cells. HAI titres of

1:40 (typically, depending on the laboratory method used) are associated with a 50% or more protection against influenza infection (27). There is no such direct correlation for neutralization titres. Further details of the HAI titres are found in the publications in this thesis.

The usual measure of seroconversion - using antibody studies as an indication of probable infection - is a four-fold or greater increase in antibody levels post-infection compared to pre-infection or acute phase samples in the same individual. This necessitates serial sampling and this may pose a challenge as it requires early blood samples being taken and follow up weeks later; this also reduces its use for individual clinical management. Two recent studies have found that for the 2009 H1N1 influenza pandemic, antibody levels increase rapidly after two weeks post infection (28,29). Both neutralization and HAI have good sensitivities of more than 80% compared to PCR although neutralization tests using microneutralization techniques are usually slightly more sensitive than HAI (28). HAI is the test used for traditional vaccine efficacy studies, and is easier and less time consuming to perform than neutralization tests. In some population-based epidemiological studies only a single post-infection sample is available in the study population. In these cases, a cut-off antibody detection level is used to indicate probable immunity and the immune proportion in the study population are then compared with banked blood samples obtained from other individuals that are used to estimate pre-existing immunity levels to obtain an overall estimated infection rate. Further details are shown and discussed in Chapter Four.

Immunofluorescence testing with direct or indirect antibody staining can also be performed in a specialized laboratory setting and yield results within four hours. It

detects viruses in the clinical samples obtained from the patient. The viral antigens are bound to monoclonal antibodies that can be influenza type, subtype, or strain specific, and illuminated using fluorescence staining of the antibodies. However, these immunofluorescence tests are in general less sensitive compared to PCR tests (30).

Rapid diagnostic tests, which are immunoassays, are also available that can yield results in less than one hour and often within minutes, and can be used as a point-of-case test. There are many different types of rapid diagnostic test kits available on the market, but their sensitivity and specificity for detecting influenza generally or specific subtypes or strains vary widely and are often poor compared to the more established methods mentioned above (30). Their use in clinical management must therefore take into account the low sensitivity, especially if clinicians would like to use them to determine the need for early treatment or isolation in individual cases. Rapid tests may instead be more useful in identifying influenza outbreaks as more suspect cases are available for testing such that the misidentification of a few cases due to the low sensitivity would not hinder the overall identification of the outbreak for public health measures to be taken.

Influenza Epidemics and Pandemics

Influenza epidemics and pandemics occur with such regularity that the resultant impact is substantial and of interest to public health workers and policy makers. The main reason for these regular epidemics and pandemics is that influenza A and B viruses lack adequate proofreading by the virus's RNA polymerase during replication of progeny RNA. This results in frequent point mutations in the surface glycoproteins and the virus accumulates new antigenic properties that are sufficiently different from

previously circulating influenza strains – this is known as antigenic drift. Individuals who have not been exposed to similar influenza strains will be susceptible to infection by the novel virus as they have no pre-existing immunity to the new strain. This results in seasonal epidemics where a proportion of the world's population does not have pre-existing immunity and is affected. During each influenza epidemic, the proportion of the population infected by the virus depends on the amount of differentiation in the genetic makeup of the new strain from previously circulating viruses, and the pre-existing immunity of the population to closely related strains. This will be discussed further in Chapter Eight. The overall impact of the epidemic will depend not only on the disease spread and infection rate, but also on the clinical severity of illness.

Pandemics are caused by the creation of a totally novel subtype or strain of influenza A virus that is substantially different from existing circulating strains such that the vast majority of the world's population does not have immunity to the virus and are susceptible to infection. This is known as antigenic shift and this can occur through direct transmission of an influenza subtype from another species (e.g. birds) with adaptation to humans which was the postulated origins of the 1918 "Spanish Flu" pandemic (31); or from genetic reassortment where genetic material from various influenza strains came together to form a new virus, which was postulated for the 1957 and 1968 pandemics (32,33). Reassortment commonly occurs when humans or pigs are co-infected with different influenza strains giving rise to a novel virus with genetic material from both strains. Reassortment can occur multiple times resulting in a virus with components from multiple strains, which was the situation leading to the creation of the 2009 H1N1 pandemic strain. This is described in further detail in later

chapters. Some of this reassorted genetic material in the novel virus allows for effective transmission among humans, while having surface proteins from different strains ensures that the new virus is different from existing circulating human strains. As most people will not have pre-existing immunity to the new strain, the virus will spread globally with high infection rates. However, the overall impact of an influenza pandemic can vary depending on clinical severity of the virus – for example, the mortality rate was very high during the 1918 pandemic compared to the much lower mortality rates of the 2009 pandemic.

This thesis explores influenza epidemics and pandemics in the tropics, especially during the 2009 H1N1 pandemic, including the spread, infection rates, and effectiveness of various interventions to reduce the spread of the virus.

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Chapter Three

Epidemic and Pandemic Influenza in Tropical Singapore

Singapore is a tropical city-state in South-East Asia, 1°15' north of the equator. Singapore covers an area of slightly more than 700 square kilometres and has a population of about five million people. It has a tropical climate with no distinct climatic seasons and is characterized by high temperatures, rainfall, and humidity. Singapore is a global transportation hub with high volumes of travel and trade, making it a melting pot of cultures and potentially vulnerable to the spread of infectious diseases. For example, the SARS (severe acute respiratory syndrome) outbreak in 2003 spread quickly from China to Hong Kong and then to Singapore. Singapore is therefore an ideal setting to study the spread and transmission of influenza.

The patterns of influenza transmission in the tropics are substantially different from those in temperate regions. In countries with temperate climates, seasonal influenza epidemics usually occur during the winter months and influenza cases surge rapidly and remain above baseline levels for about six to eight weeks (1). This is different in the tropics which lack well-defined climatic seasons. Surveillance data shows that Singapore has a high baseline incidence of influenza and other respiratory diseases throughout the year, and seasonal influenza epidemics in Singapore usually occur during the second and fourth quarter of the year, often with more than one distinct peak of influenza activity each year (2,3). Although the pattern of influenza outbreaks in tropical regions differs from temperate countries, the impact of influenza remains similar. In Singapore, about 20% of the population is affected by influenza annually, resulting in an estimated 500,000 physician visits and 300,000 work days lost (4). A study in Singapore showed that the excess mortality attributable to influenza was of similar magnitude in tropical Singapore (14.8 per 100,000 person-years), sub-tropical

Hong Kong (16.4 per 100,000 person-years), and temperate United States (19.6 per 100,000 person-years) (5). Although there is general awareness of the impact of these diseases globally, there is a lack of data on respiratory diseases in tropical regions.

To address these gaps, I previously published two papers examining the impact of the three pandemics of the 20th Century in Singapore (6,7). The studies showed that the local epidemics of novel pandemic influenza viruses in Singapore occurred early during the global pandemic's course, and resulted in significant mortality. The studies showed that tropical Singapore was not spared from the impact of previous pandemics, regardless of their geographical origin. To build upon this evidence, the following study aims to provide additional evidence on the impact of seasonal epidemic influenza in tropical Singapore, and on the relationship and timing between epidemics in Singapore and in other countries across the world. This will show that influenza affects tropical regions substantially and consistently, and that attention should be paid to reduce the burden of disease in the tropics.

Study 1

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Influenza Excess Mortality from 1950–2000 in Tropical Singapore

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Abstract

Introduction: Tropical regions have been shown to exhibit different influenza seasonal patterns compared to their temperate counterparts. However, there is little information about the burden of annual tropical influenza epidemics across time, and the relationship between tropical influenza epidemics compared with other regions.

Methods: Data on monthly national mortality and population was obtained from 1947 to 2003 in Singapore. To determine excess mortality for each month, we used a moving average analysis for each month from 1950 to 2000. From 1972, influenza viral surveillance data was available. Before 1972, information was obtained from serial annual government reports, peer-reviewed journal articles and press articles.

Results: The influenza pandemics of 1957 and 1968 resulted in substantial mortality. In addition, there were 20 other time points with significant excess mortality. Of the 12 periods with significant excess mortality post-1972, only one point (1988) did not correspond to a recorded influenza activity. For the 8 periods with significant excess mortality periods before 1972 excluding the pandemic years, 2 years (1951 and 1953) had newspaper reports of increased pneumonia deaths. Excess mortality could be observed in almost all periods with recorded influenza outbreaks but did not always exceed the 95% confidence limits of the baseline mortality rate.

Conclusion: Influenza epidemics were the likely cause of most excess mortality periods in post-war tropical Singapore, although not every epidemic resulted in high mortality. It is therefore important to have good influenza surveillance systems in place to detect influenza activity.

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Introduction

Tropical regions have been shown to exhibit different influenza seasonal patterns compared to temperate regions. While temperate countries have a single annual epidemic during winter, influenza in the tropics spreads throughout the year, with two annual peaks having been described for Singapore, a globally-connected tropical city [1,2]. However, while the seasonality differs from temperate countries, mortality from influenza activity in tropical Singapore is comparable to temperate and sub-tropical countries such as the United States and Hong Kong [3]. The three 20th century influenza pandemics in Singapore were also associated with substantial excess deaths when compared against baseline mortality rates in surrounding years [4].

Long time-series data on successive influenza seasons have been used to highlight and quantify the burden of disease attributable to influenza in temperate countries [5–8]. In addition, such data has been used to grade the severity of different epidemic influenza seasons as well as specific influenza sub-types and strains [9–11]. However, there are few equivalent studies on the burden of annual influenza epidemics in the tropics, and the relationship between tropical influenza epidemics compared with other regions [12]. There is a scarcity of data on tropical influenza, due to the lack of clear seasonality and virological data to identify periods of influenza activity and its associated impact on mortality.

Singapore is a tropical island city-state in South-East Asia. Being a globally-connected city, it provides a representation of the spread of influenza in the tropics. In addition, Singapore has good consistent records of mortality statistics, and had been routinely isolating influenza viruses for surveillance since 1972 (as a nationwide study in 1972 and 1973, and as a national surveillance programme from 1974 onwards) [1]. In this study, we explore the possible links between excess mortality from 1950 to 2000 in the post-World War Two era in Singapore and influenza epidemics. This time-period also included almost 30 years of influenza virological surveillance data. We use this data to demonstrate the

clear correlation between influenza epidemic periods and excess mortality, and highlight the burden and timing of prominent influenza epidemics in tropical Singapore.

Materials and Methods

Data on monthly national all cause mortality and population size was obtained from 1947 to 2003 from the Registry of Births and Deaths, Singapore through the Department of Statistics, Singapore–the governmental agency responsible for collection, verification, and maintenance of national statistics. Monthly mortality rates were calculated from this data.

To determine the excess mortality for each month from 1950 to 2000, we used a moving average analysis which has proven appropriateness due to the lack of distinct seasonal mortality patterns in Singapore [4]. Unlike temperate regions, where methods relying on seasonal variation such as that used by Serfling are commonly used, we assumed that monthly mortality in Singapore exhibited a secular trend without seasonal components. We therefore used a moving average analysis for each month constructed using data from 3 years before and 3 years after the month (excluding the month itself) to calculate the predicted mean and 95% confidence intervals for the expected mortality for that month. Months previously known to be affected by the 1957 and 1968 pandemics (May 1957, and August and September 1968) were excluded to eliminate inflation of confidence intervals in the periods surrounding those months. The moving averages formed the entire baseline mortality rate with 95% confidence intervals across 50 years from 1950 to 2000. Excess deaths were calculated as the actual mortality rate on record minus the moving average baseline rate. Months for which the mortality rate exceeded the 95% confidence intervals were considered as those with significant excess deaths, and used to highlight possible influenza epidemic periods. The analyses were performed in Stata 10.0 for Windows (Stata Corp., College Station, TX, USA).

Data on influenza virological surveillance was obtained from the Department of Pathology at the Singapore General Hospital, which is the World Health Organization (WHO) National Influenza Centre (NIC) in Singapore since 1972 [13]. The surveillance programme tracks influenza activity year-round through virus isolation and identification from respiratory samples collected from patients attending government outpatient clinics for influenza-like symptoms, as well as from patients in hospitals and private clinics. Strain characterization was performed at the WHO Collaborating Centres for Reference and Research on Influenza in the USA, UK and Australia, and at the NIC. The data included records of predominant strains and their periods of circulation in Singapore, which were available from 1972 onwards. The proportion of respiratory illness samples positive for influenza was available from 1972 to 1993, and the breakdown on the percentage of samples positive for influenza A (H1N1), (H3N2), and influenza B was available from 1994 onwards. These proportions were compared to mortality rates to determine if periods with excess mortality corresponded to increases in influenza isolates.

In addition, we performed a search of serial annual government reports from the Department of Health, Singapore from 1950 to 1965 (before Singapore gained independence), the Ministry of Health and the Ministry of the Environment from 1965 onwards (post-independence), peer-reviewed journal articles, and press articles from The Straits Times (the main and only English newspaper across all the years). This was done for all the months where significant excess mortality occurred, to determine if there were reports suggestive of influenza epidemics. This provided additional evidence of the known influenza outbreaks, and the recorded burden of these epidemics.

Results

The annual number of deaths from 1950 to 2000, and excess deaths where mortality exceeded the upper limit of the 95% confidence interval is shown in Figure 1A. Apart from the known pandemics of 1957 and 1968, which resulted in substantial mortality, there were 20 other time points in which there was significant excess mortality.

Excluding the two pandemics, there were 8 periods with excess mortality before 1972. Of these, 2 periods coincided with newspaper reports of increased pneumonia deaths. There were 3 weeks in August 1953 where pneumonia was the mentioned as the main cause of deaths in newspaper reports (weeks ending August 8 with 23 deaths, August 15 with 30 deaths, and August 22 with 31 deaths) [14,15]. In August and September 1951, pneumonia was also the main cause of deaths for the weeks ending August 18 (29 deaths), August 25 (30 deaths), September 2 (35 deaths) [16–18]. In end-September 1951, there was also an increase in pneumonia cases in Malaysia (a country North of Singapore also under the administration of the British Empire at the time) [19].

Figure 1B shows the significant excess mortality periods and the % of respiratory illness sample isolates positive for influenza from 1972 onwards when virological data became available. There were 12 periods with significant excess mortality post 1972 for which virological data was available. Figure 2 shows the excess mortality and corresponding influenza virological surveillance for these periods. Of the 12 periods with excess mortality post 1972, only one (1988) did not coincide with a temporal increase in the percentage of respiratory illness samples that were positive for influenza, although only 8 of the 12 periods were explicitly labeled as influenza epidemics or outbreaks on government records.

Table 1 lists all time periods with significant excess mortality, along with periods described as influenza epidemics or outbreaks on government records. Over the 29 years (1972 to 2000) for which influenza surveillance records are described, there were 21 influenza epidemic periods, and an additional 4 periods where significant excess mortality was observed (three of which corresponded to an increase in virological activity). Of the 12 periods of significant excess mortality post-1972, 5 of the peak excess mortality months occurred in the month of May, followed by 4 in January, and 1 each in March, June, and July. Of the 13 epidemic periods which did not result in significant excess mortality, 5 epidemic peaks occurred in the month of May, with 2 peaks each in January and July, and one each in Mar, April, June, and October. The month of May also dominated in the 8 non-pandemic significant excess mortality periods pre-1972, with 4 occurring in that month, and 1 each in January, August, September, and October. Overall, there were more reported increases in May compared to all other months.

Influenza epidemics often accompanied the introduction of new influenza antigenic variants, and new influenza variants also often gave rise to second epidemic waves. The spread of H1N1 following its re-emergence in late 1977 followed such a pattern, with the first wave causing epidemics from Dec 77 to Feb 78 (as A/USSR/1/77), followed by a second wave which passed through Singapore from Sep 78 to Oct 78 (as A/Brazil/11/78). In most cases, second waves had less mortality than the first, the exception being A/England/42/72, where both epidemic waves caused substantial excess mortality, with the first wave which peaked in Jul 72 being milder than the second wave which peaked six months later in Jan 73.

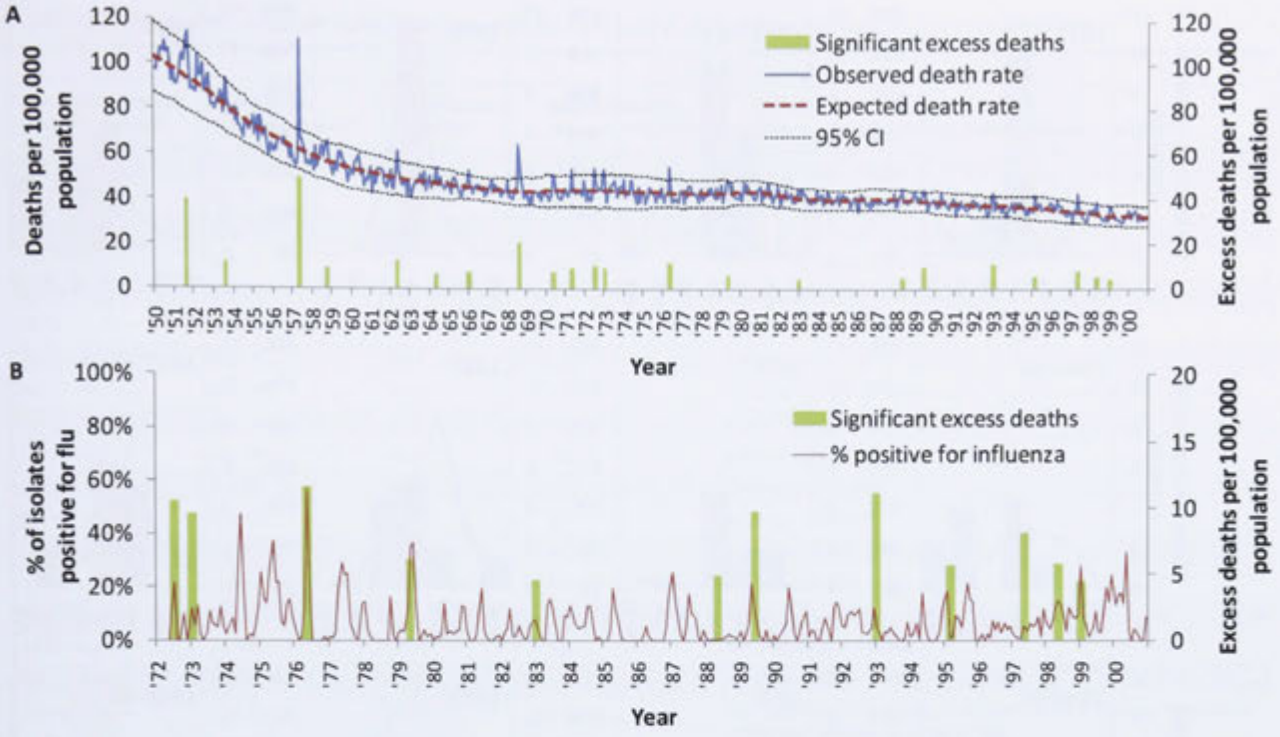

Figure 1. Periods of significant excess deaths in tropical Singapore*. Panel A–Excess deaths compared to overall deaths, 1950 to 2000. Panel B–Excess deaths compared to positive influenza positive, 1972 to 2000. *Significant excess mortality which occurred over 2 contiguous months (August to September 1951, June to July 1989, and December 1992 to January 1993) was summed to allow for comparisons of overall epidemic magnitude.
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Epidemic periods which did not give rise to significant excess mortality caused lower overall excess mortality, although positive deviations from baseline mortality were detectable for most periods for which epidemic activity had been reported (Figure 3). Temporal peaks in mortality either corresponded to or lagged by one month the peaks in percentage of respiratory illness isolates positive for influenza.

Table 2 compares the epidemic timing and excess mortality attributable to prominent epidemics (the two pandemics (1957 and 1968), H1N1 emergence in 1977, and 1951 where pneumonia deaths contributing substantially to overall mortality) to the 10 time periods with most excess mortality after 1972 when virological data became available. With the exception of the A/Port Chalmers/1/73 (H3N2) epidemic in May 1974, 9 of the 10 periods also qualified as periods of significant excess mortality. Excess deaths in Aug-Sep 1951 (46 excess deaths per 100,000) were comparable to the 1957 pandemic (54 excess deaths per 100,000) while the H3N2 pandemic of 1968 caused half as much excess mortality. Estimates for the most severe influenza epidemic seasons ranged from 7.0 to 15.8 excess deaths per 100,000 population. With the exception of B/Singapore/222/79, H3N2 influenza activity featured in all the other 9 time periods. The re-emergence of H1N1 influenza as A/USSR/1/77 caused relatively mild mortality with only 3.5 excess deaths per 100,000 population; this was compared to a mean of 11.3 for the 10 episodes featured in Table 2, and 6.9 for all the periods listed in Table 1. Of the 10 periods with the most severe excess mortality, half occurred in the month of May; three others occurred in January, with one each in Jun and Jul.

Most of the key antigenic drift variants which caused severe epidemics in Singapore also caused epidemics elsewhere (Table 2). In 1951, 1972/3, 1976, and 1998/99, the periods of high mortality corresponded to some of the highest non-pandemic mortality periods [20]. However, the relative mortality of individual epidemics vary in different countries. The 1975–76 epidemic in England and Wales due to A/Victoria/3/75 (H3N2) had an excess mortality of 60.84/100000 (37), which was much higher than similar epidemics in Singapore and the US (10). In Singapore and the US, most of the outbreaks caused by the similar viruses in similar time period had generally comparable excess mortality rates (see Table 2). However, the A/Sydney/5/97 (H3N2) epidemics in the US had a much higher mortality of 26.82 and 26.10 per 100,000 population in the winters of 1997–98 and 1998–99 respectively (11). This was higher compared to that of Singapore and as well as many previous epidemics in the US (11). The timing of occurrence in different countries also varies. The first wave of infections with A/England/42/72 (H3N2)-related viruses peaked in Singapore in July 1972; while infections peaked about the same time in Australia in August 1972, excess mortality was not markedly high [21]. In the Northern hemisphere winter of 1972 to 1973, relatively severe epidemics of A/England/42/72 (H3N2) were noted on both sides of the Atlantic Ocean [6,22]. Singapore also experienced a severe second wave which peaked in Jan 73. For influenza A/Port Chalmers/1/73 (H3N2), outbreak reports in New Zealand dated to Sept 1973 [23] but the first wave in Singapore only peaked in May 1974, and related viruses did not cause substantial mortality in Northern Hemisphere countries like the USA until the winter of 1974 to 1975 [6]. The epidemic of B/Singapore/222/79 in May 1979 also heralded the epidemic in the USA in the winter of 1979 to 1980 [24]. Conversely, A/Victoria/3/75 (H3N2) related viruses caused a severe influenza season in the USA and England and Wales in the winter of 1975 to 1976

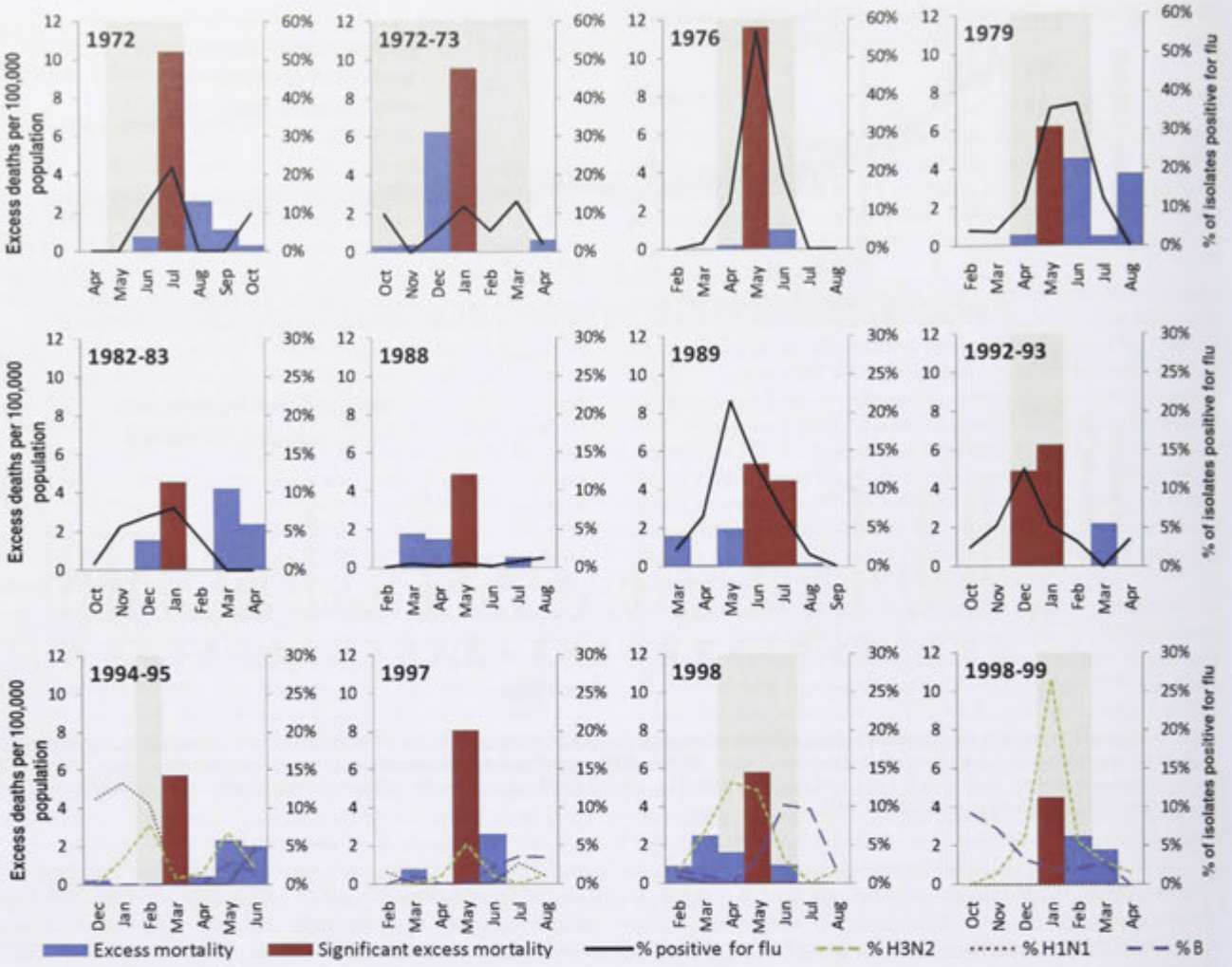

Figure 2. Periods with significant excess deaths in Singapore and positive influenza isolates, 1972 to 2000*. *Areas shaded in grey correspond to official reports of influenza epidemics during the time period.
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with excess mortality of 11.39 and 60.84 per 100,000 population respectively [6,25] before the first wave spread through Singapore in April to June 1976. While A/Sydney/5/97 (H3N2) infections were reported in Australia in the mid-1997 influenza season, this was followed by the spread in the Northern hemisphere [11,26] before major epidemics in Singapore and some other countries [27] in the earlier half of 1998.

Discussion

Influenza possibly accounted for the majority of time periods with significant excess mortality in tropical Singapore in the post-war years, including the 3 highest mortality records in 1951, 1957, and 1968. Although most pre-1972 significant excess mortality months lacked documented evidence of influenza epidemics, two periods (August and Sept 1951, and August 1953) coincided with media reports of pneumonia deaths, and an additional four periods occurred in May–the month with the most number of known influenza epidemic peaks. This suggests that influenza probably resulted in more excess mortality than any other variable cause. The second highest excess mortality in 1951 was only slightly less than the 1957 pandemic and 70% higher than the 1968 pandemic. This coincided with 3 consecutive newspaper reports of pneumonia dominating mortality [16–18], and excess pneumonia deaths correlating with increased all-cause mortality are known to occur during influenza epidemics [10]. The year 1951 also saw major influenza outbreaks caused by the influenza A (H1N1) virus, with higher mortality and transmissibility in England and Wales and Canada than the 1957 and 1968 pandemics [28]. In Liverpool, the supposed epicenter of the 1951 epidemic, severity was higher than the 1918 pandemic [29]. Our data strongly suggests that the burden of the 1951 epidemic was not restricted to temperate regions but also affected tropical countries like Singapore and Malaysia.

In contrast, the large dengue outbreaks in the 1990s and 2000s, which where constantly in the media reports, resulted in much fewer deaths [30–32]; while the worst industrial accident in Singapore's history, the Spyros oil tanker explosion, killed only 76 people. The number of deaths attributable to each significant influenza epidemic was much higher than that caused by any other known man-made or natural cause during the same period. Based on this observation, it is therefore of public health and socio-economic importance to have good surveillance and prevention programs against seasonal influenza. Future measures should include promotion of annual

Table 1. All reported influenza epidemics and months with excess mortality in Singapore, 1972 onwards.

Reported influenza epidemic period	Peak excess mortality month*	Overall excess mortality†	Excess mortality rate per 100,000†	Dominant influenza strains during epidemic period
May 72–Jul 72	Jul 72	294.7	13.8	A/England/42/72 (H3N2), 1st wave‡
Oct 72–Mar 73	Jan 73	341.2	15.8	A/England/42/72 (H3N2), 2nd wave‡
May 74–Jul 74	May 74	218.1	9.9	A/Port Chalmers/1/73 (H3N2), 1st wave
Nov 74–Feb 75	Jan 75	3.5	0.2	A/Port Chalmers/1/73 (H3N2), 2nd wave
Apr 75–Jun 75	May 75	54.9	2.4	A/Scotland/840/74 (H3N2)
Jul 75–Jul 75	Jul 75	12.4	0.6	B/Hong Kong/5/72
Apr 76–Jun 76	May 76	291.7	12.8	A/Victoria/3/75 (H3N2)‡
Apr 77–Jul 77	May 77	160.0	6.9	A/Victoria/3/75 (H3N2), A/Texas/1/77 (H3N2) and B/Hong Kong/5/72
Dec 77–Feb 78	Jan 78	82.4	3.5	A/USSR/1/77 (H1N1)
Sep 78–Oct 78	Oct 78	59.4	2.5	A/Brazil/11/78 (H1N1)
Apr 79–Jun 79	May 79	264.2	11.2	B/Singapore/222/79‡
Apr 80–Jun 80	May 80	121.4	5.1	A/Texas/1/77 (H3N2)
May 81–Jun 81	Jun 81	121.4	4.9	A/England/333/80 (H1N1)
-	Jan 83	162.4	6.1	A/Philippines/2/82 (H3N2)‡
May 83–Jul 83	May 83	93.0	3.5	A/Chile/1/83 (H1N1)
Jul 84–Sep 84	Jul 84	136.4	5.0	A/Philippines/2/82 (H3N2)
Apr 85–Jun 85	Apr 85	115.5	4.2	A/Philippines/2/82 (H3N2)
Mar 86–May 86	Mar 86	84.7	3.1	A/Switzerland/79/85 (H1N1), A/Dunedin/27/84 (H1N1), A/Victoria/7/83 (H1N1) and A/Singapore/6/86 (H1N1)
-	May 88	179.3	6.4	A/Victoria/7/87 (H3N2), A/Sichuan/2/87 (H3N2), A/Sydney1/87 (H3N2) and B/Victoria/2/87‡
-	Jun 89	339.2	11.7	A/Shanghai/11/87-like (H3N2) and A/OMS/5389/88-like (H3N2)‡
Dec 92–Jan 93	Jan 93	361.0	11.2	A/Beijing/32/92 (H3N2)‡
Feb 95–Mar 95	Mar 95	212.9	6.2	A/Taiwan/86 (H1N1), A/Texas/36/91 (H1N1)‡
-	May 97	398.0	10.7	A/Wuhan/359/95 (H3N2)‡
Apr 98–Jun 98	May 98	324.1	8.4	A/Sydney/5/97 (H3N2), 1st wave‡
Jan 99–Feb 99	Jan 99	275.2	7.0	A/Sydney/5/97 (H3N2), 2nd wave‡

*Month with highest excess mortality during a reported influenza epidemic period, or month with highest excess mortality in a period with significant excess mortality.
†Sum of positive deviations from the expected mortality for three-month period centered around the month with peak excess mortality; excess mortality rate is derived using estimates for total Singapore population during that period.
‡Periods with significant excess mortality.
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influenza vaccination, and early epidemic identification and virological surveillance to allow judicious use of measures such as anti-viral treatment of influenza during epidemic periods. Vaccine utilization in Singapore has hitherto been poor (<0.5% of the population), making vaccine mismatch less relevant [3]. In concert with promoting seasonal vaccination programs, studies are needed to explore vaccine match to tropical epidemic strains. Other studies should also be performed to validate the utility of different surveillance systems, and determine the cost-effectiveness of surveillance and intervention programs.

While our simple algorithm for flagging months with significant excess mortality appears to have a high specificity for detecting periods of influenza activity, not all significant epidemics were identified by the algorithm. For instance, the first wave of the A/Port Chalmers/1/73(H3N2) virus which peaked in May 1974 caused substantial excess mortality (Table 2), but did not exceed the 95% confidence limits for that period, possibly because it was flanked by several epidemics of equal or greater severity (A/England/42/72 in Jul 1972 and Jan 1973, and A/Victoria/3/75 (H3N2) in May 1976). Some epidemics may also have had high morbidity with low mortality such as the A/USSR/90/77(H1N1) strains, which re-emerged in November 1977. In Singapore, infections were reported among military personnel in mid-December 1977 and spread quickly, with the epidemic peaking in January 1978 and lasting until February 1978. Government reports indicated that although outpatient attendances for upper respiratory tract infections (URTIs) doubled in January 1978, there was no corresponding increase in pneumonia and influenza (P&I) deaths [1]. As the virus affected mainly children and young adults, the relative sparing of older populations might explain the lower overall mortality of the 1977 H1N1 epidemic. Age-related immune protection in older individuals (2009) might also cause the influenza A H1N1-2009 pandemic to have a lower overall mortality rate (US CDC, 2009).

Our data also suggests the importance of correlating viral characteristics with mortality. As the 1951 epidemic suggests, epidemic seasons around that time were relatively mild in the United States in terms of morbidity and mortality, but were far more severe in Canada, England and Wales [28] and Singapore and Malaysia. In addition, in the post-1972 period, new influenza variants were noted to cause epidemics in Singapore, at times preceding (e.g. B/Singapore/222/79) and at other times following

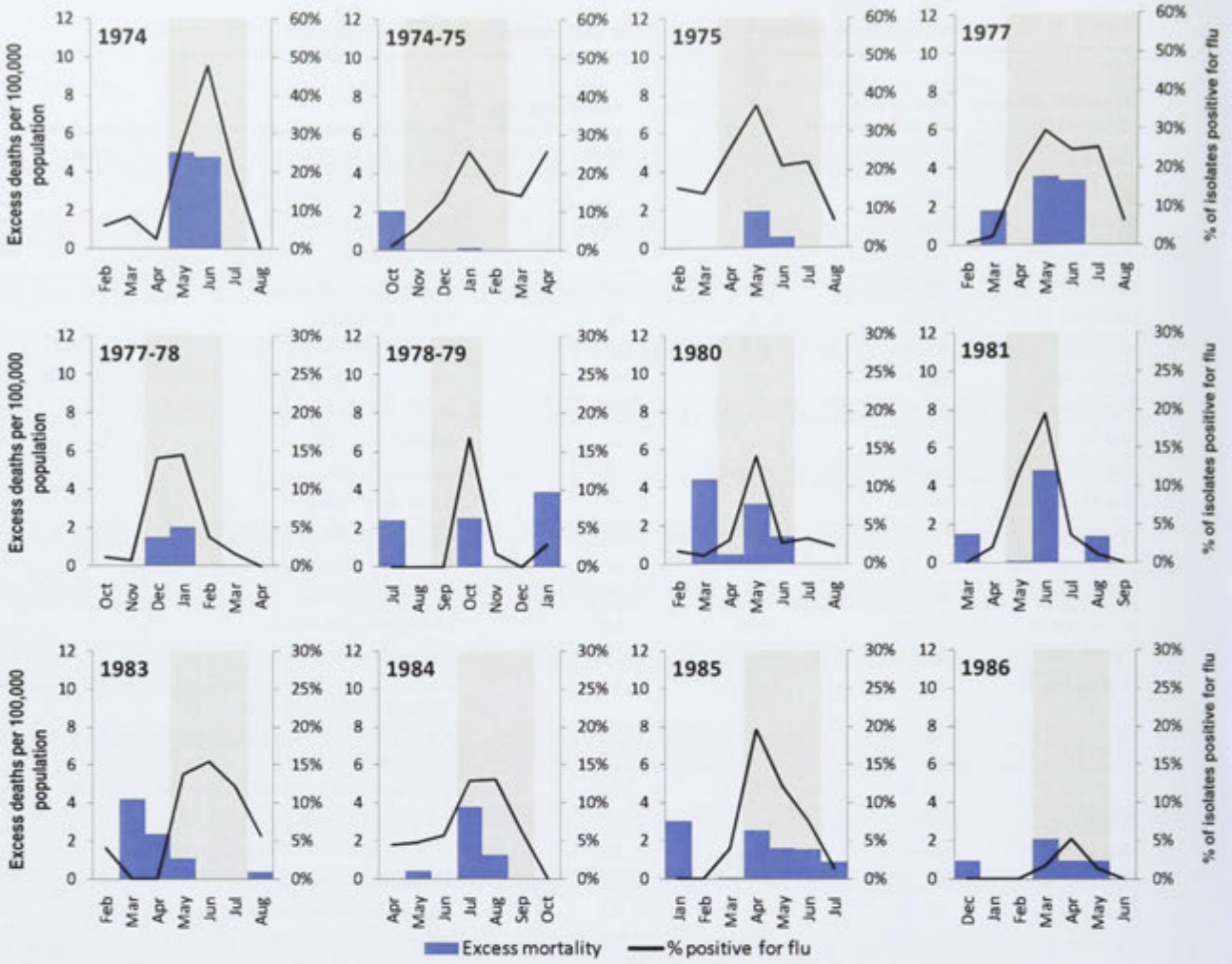

Figure 3. Additional epidemic periods in Singapore, with corresponding excess deaths and positive influenza isolates, 1972 to 2000*. *Areas shaded in grey correspond to official reports of influenza epidemics during the time period.
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outbreaks and epidemics in other countries (e.g. A/Sydney/5/97). A systematic review comparing the burden and relative timing of different influenza seasons with a focus on identifiable strains could yield deep insights into how influenza circulates globally, with important ramifications for vaccine strain selection.

For now, the reasons for the asynchronous nature of the timing of global influenza epidemics and their differential burden of disease in different regions, as well as the driving force behind the timing of influenza epidemics in Singapore remains a mystery. Although there is little climatic seasonal variation in Singapore, this study shows that influenza seasons tend to occur from April to July and November to February, with May having most epidemics and peak excess mortality. The observed seasonality could be due to subtle differences in climate, new strains developing in tropical areas during the corresponding periods, or the spread of viruses from temperate regions. Herald waves which occur during the spring in Northern Hemisphere temperate countries [24] and taper off during the summer months, while failing to propagate in temperate countries, could go on to cause epidemics in tropical countries which are receptive to influenza viruses throughout the year.

Limitations of our study include the use of monthly all cause data which may under-estimate the burden of epidemics that straddle 2 to 3 months, as mortality split across more than 1 time period may not appear significant compared to epidemics where mortality is concentrated within the time period of analysis. The moving average calculation of the confidence intervals also means that less severe epidemics may be missed if flanked by more severe epidemics, as was the case for the 1974 epidemic caused by influenza A/Port Chalmers/1/73. Data resolution is another issue as there was no age-specific or influenza-specific data available across all 50 years. The accuracy of disease burden estimates is also difficult to assess, and different methods should be used for comparison. For example, a study in Hong Kong Island by Chiu and colleagues [33] determined influenza hospitalization rates through virological testing of hospitalized respiratory cases. Virologic data is also imperfect, as it represents only % of samples tested and not the actual size of the epidemic. As such, future studies will compare the range of estimates obtained through influenza-specific mortality, and different data sources such as primary care data and virologic testing of hospitalized or fatal cases where available. Finally, comparisons of disease burden across countries are difficult due to different methods used and future studies should analyze global data simultaneously.

Nevertheless, this study shows that crude mortality estimates can be sufficient to signal the most significant influenza epidemics, and

Table 2. Ten most severe recorded influenza epidemic seasons in Singapore from 1972 to 2000 and selected countries with similar epidemics during the period, in comparison with selected influenza pandemics and epidemics.

	Excess mortality per 100,000	Month with excess peak mortality	Selected countries affected by the same virus during the same period, with timing of epidemics in parentheses. Excess all cause mortality per 100,000 are shown for selected outbreaks in the US and England & Wales where data is available
Ten most severe recorded influenza epidemic seasons			
A/England/42/72 (H3N2), 1st wave	13.8	Jul 1972	India (1971–72), Nepal (1972–1973) [34] Australia (August 72) [20] England & Wales (1972–73) [21] United States (1972–73) [6,10]–Excess mortality 9.0
A/England/42/72 (H3N2), 2nd wave	15.8	Jan 1973	As above.
A/Port Chalmers/1/73 (H3N2), 1st wave	9.9	May 1974	Port Chalmers, New Zealand (1973) [22] Nigeria (1974) United States (1974–75) [6,10] - Excess mortality 7.0 Houston, United States (1974–75) [35]
A/Victoria/3/75 (H3N2)	12.8	May 1976	United States (1975–76) [6,10] - Excess mortality 11.4 Houston, United States (1976) [36,37] England & Wales (1975–76) [25] - Excess mortality 60.8
B/Singapore/222/79	11.2	May 1979	United States (1979–80) [6,10] - Excess mortality 7.6 Houston, United States (1979–80) [35] England & Wales (1978–79) [25] - Excess mortality 16.3
A/Shanghai/11/87-like, A/OMS/5389/88-like (H3N2)	11.7	Jun 1989	USA (1989–90) (A/Shanghai/11/87) [38] - Excess mortality 4.2 [6,10] Poland (1990) (A/OMS/5389/88) [39] England & Wales (1988–89) [25] - Excess mortality 20.0
A/Beijing/32/92 (H3N2)	11.2	Jan 1993	USA (1991–92) [10] - Excess mortality 16.7 Netherlands (1993–94) [40] Ontario, Canada (1993–94) [41] Lasi, Romania (1993–94) [42]
A/Wuhan/359/95 (H3N2)	10.7	May 1997	USA (1996–97) [11] - Excess mortality 25.9 Pune, India (Jan–Feb 1996) [44] Poland (Feb 1997) [45] Thailand (Jul–Aug 1997) [46]
A/Sydney/5/97 (H3N2), 1st wave	8.4	May 1998	USA (1997–98) [11,47] - Excess mortality 26.8 Australia (1997) [24] South Africa (1998) [25]
A/Sydney/5/97 (H3N2), 2nd wave	7.0	Jan 1999	USA (1998–99) [11] - Excess mortality 23.1
Other epidemics and pandemics of note in Singapore			
1951 influenza epidemic strain (H1N1)	46.4	Sep 1951	
Asian influenza pandemic (H2N2)	54.4	May 1957	
Hong Kong influenza pandemic (H3N2)	28.0	Aug 1968	
A/USSR/1/77 (H1N1)	3.5	Jan 1977	

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can be easily applied to countries where data of finer resolution may be lacking. International comparisons of East Asian and tropical Southeast Asian countries should be considered, particularly at new variants in view of recent phylogenetic work which suggests that the region may be critical in the genesis of new H3N2 influenza strains [34].

Influenza epidemics were the likely cause of most of the excess mortality periods in post-war tropical Singapore, although not every influenza epidemic resulted in high mortality. It is important to have good public health programs in place to detect influenza activity. Such programmes, along with appropriate public health interventions like vaccination and judicious antiviral use, could potentially reduce the burden of seasonal or pandemic influenza.

Author Contributions

Conceived and designed the experiments: VJL KTG YSL MICC. Performed the experiments: VJL JY JO SPC MICC. Analyzed the data: VJL JY JO SPC MICC. Contributed reagents/materials/analysis tools: VJL JY KPC RTPL KTG MICC. Wrote the paper: VJL JY JO KPC RTPL YSL MICC.

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Chapter Four

2009 H1N1 Influenza in Singapore and Estimating Overall Infection Rates

As we move into the 21st Century, one of the questions that many public health decision makers were focused on was not if but when the first influenza pandemic of the new Century would arrive. Many countries had developed influenza pandemic preparedness and response plans for this eventuality, and had spent considerable resources on various preparedness programs. The world did not have to wait very long for the next pandemic as a novel H1N1 influenza virus was reported by the WHO on 24 April 2009. The pandemic spread quickly from it's origins in Mexico in early 2009 to the United States, and soon after to Canada, New Zealand, Israel, several European countries by 1 May 2009, and then to the rest of the world, resulting in the first influenza pandemic of the 21st Century (1).

As with previous pandemics, Singapore was yet again affected by the 2009 pandemic, although the arrival of the pandemic in Singapore occurred about a month after it was first reported globally. The first imported case of 2009 pandemic H1N1 influenza was detected in Singapore on 26 May 2009 and reported the next day (2). From May to 9 July 2009, there were 467 imported influenza cases of which the most frequent country of travel was Australia, the Philippines, Indonesia, Thailand, and the United States (3). Transmission in the local community (detection of an unlinked local case) was first reported on 18 June 2009 (4).

Although Singapore is a travel hub, it did not identify the first imported case for several weeks after other similar hubs in the region such as Hong Kong or Melbourne were affected. This occurred in spite of the high rates of travel from these affected regions and the substantially increased levels of vigilance for the novel virus in Singapore. For example, in Hong Kong, a city similar to Singapore, the first imported

case was reported on 1 May 2009 (5). The reasons for the later detection in Singapore are unclear and it is likely that milder cases could have been imported or that some cases had evaded detection (3). At the same time it may also be possible that the delay was due to the measures implemented by the Singapore Government at that time, although there is no available scientific evidence that these measures were effective. The initial containment measures included temperature screening via thermal scanners at all entry points to Singapore, testing and isolation of all 2009 H1N1 cases, distribution of health alert notices to all arriving individuals, and temporary visa requirements for visitors from Mexico from 2 to 12 May. These served to increase the awareness of the measures by arriving passengers (6), and perhaps reduced the likelihood of ill travelers coming to Singapore.

At the same time, there was also a substantial delay between the first reported imported case and the first reported local transmission case in Hong Kong (5), which was similar to the experience in Singapore. This could have been due to the initial measures taken by the two governments such as isolation of confirmed cases in hospital, contact tracing and quarantine of contacts, anti-viral prophylaxis for contacts, and public education (5,6). In addition, the school holidays in Singapore started on 30 May 2009 and lasted until 28 June 2009, and may have contributed to the reduction in spread. However, it is also likely that some community spread could have occurred before the first reported case of local transmission (3).

Nevertheless, after the first report of local transmission, the virus quickly spread across the country in a major epidemic wave that peaked in early August 2009 and subsided by September 2009. Due to the rapid spread of the virus globally and within

Singapore, there was an interest from policy makers to determine the infection rate from the epidemic, and the associated severity rates in terms of severe cases requiring hospitalization or ventilator support, or death. The infection rate was especially important as it represented the extent of spread and possible immunity against subsequent waves of the same influenza strain, and is necessary as a denominator to determine the severity rates. The latter point cannot be overstated, as basing the denominator solely on laboratory positive cases or similar methods will substantially overestimate the severity of the disease, as shown in the early weeks of the 2009 H1N1 pandemic from the initial data coming out of Mexico (7).

However, it is difficult to estimate infection rates from influenza due to the non-specific clinical presentation which as previously mentioned is very similar to other ILIs, and the fact that influenza often presents as sub-clinical or asymptomatic infection which is difficult to detect. One of the methods that is often used to determine the overall infection rate is serological studies which determine the increase in antibody titers to the particular influenza strain. During the 2009 pandemic, many countries worldwide used such studies with different study designs to estimate the local infection rates (8). Although this is a fairly accurate and consistent measure used to determine infection in influenza and other diseases, it is resource intensive and difficult to perform to ensure good representation of the population even in the best of situations. In addition, there is a time delay that is inherent for serological studies due to the time needed for serological conversion to occur in the infected individual, and the testing thereafter.

At the same time, many countries have good existing syndromic surveillance systems, or are able to set up these systems quickly during the lead-in to the local epidemic. These systems may provide good estimates of infection rates, but an expansion factor must be considered to include cases that are not picked up by these syndromic surveillance systems.

The following study explores this concept through the use of four different methods to estimate the infection rate of the 2009 H1N1 influenza epidemic in Singapore. This is made possible due to the various studies that have been done in the local setting, some of which have been showcased in this thesis, and the single first epidemic wave which provided an easier characterization of the start and end of the wave. The results from this study will allow policy makers and public health professionals worldwide to design surveillance systems that can provide important data during future epidemics and pandemics.

Study 2

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Practice of Epidemiology

Comparability of Different Methods for Estimating Influenza Infection Rates Over a Single Epidemic Wave

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Estimation of influenza infection rates is important for determination of the extent of epidemic spread and for calculation of severity indicators. The authors compared estimated infection rates from paired and cross-sectional serologic surveys, rates of influenza like illness (ILI) obtained from sentinel general practitioners (GPs), and ILI samples that tested positive for influenza using data from similar periods collected during the 2009 H1N1 epidemic in Singapore. The authors performed sensitivity analyses to assess the robustness of estimates to input parameter uncertainties, and they determined sample sizes required for differing levels of precision. Estimates from paired seroconversion were 17% (95% Bayesian credible interval (BCI): 14, 20), higher than those from cross-sectional serology (12%, 95% BCI: 9, 17). Adjusted ILI estimates were 15% (95% BCI: 10, 25), and estimates computed from ILI and laboratory data were 12% (95% BCI: 8, 18). Serologic estimates were least sensitive to the risk of input parameter misspecification. ILI-based estimates were more sensitive to parameter misspecification, though this was lessened by incorporation of laboratory data. Obtaining a 5-percentage-point spread for the 95% confidence interval in infection rates would require more than 1,000 participants per serologic study, a sentinel network of 90 GPs, or 50 GPs when combined with laboratory samples. The various types of estimates will provide comparable findings if accurate input parameters can be obtained.

epidemics; estimation; infection; influenza, human; population surveillance; serologic tests; statistics as topic

Abbreviations: BCI, Bayesian credible interval; GP, general practitioner; HAI, hemagglutination inhibition; ILI, influenzalike illness; RT-PCR, reverse-transcriptase polymerase chain reaction.

Assessing the spread and severity of influenza epidemics is necessary to calibrate response and mitigation strategies (1). The World Health Organization and many individual countries have made substantial investments to measure epidemic indicators. One important indicator is the epidemic's infection rate, which is crucial to quantify overall morbidity and to obtain an accurate denominator for calculating complication and mortality rates used to classify severity; the latter, in turn, guides prioritization of interventions for mitigating epidemic severity.

The 2009 influenza pandemic showed the urgency of such assessments for activation of appropriate responses, especially early in a pandemic. Because complete case counts are not feasible (2), during the 2009 epidemic public health officials in many countries attempted to estimate infection rates using whatever data were available. This included estimating clinical attack rates from influenza like illness (ILI) surveillance, determining infection rates through serologic surveys, and even using nontraditional methods such as Internet searches (3, 4). However, existing data collection plans are vital, since extrapolating from ILI surveillance necessitates estimating rates of primary-care consultation among influenza cases, which are influenced by population health-care-seeking behaviors (5), while serologic surveys require substantial planning and laboratory support (6).

With myriad estimation methods in use, it is important to determine their comparability and stability to misspecification of input parameters, to allow better interpretation of estimates over different countries and successive influenza epidemics. In this study, we answered these questions by comparing results of different methods in a single setting.

MATERIALS AND METHODS

To illustrate the different methods used worldwide to estimate infection rates during the 2009 H1N1 influenza pandemic, we performed a literature search with the PubMed search engine (US National Library of Medicine), spanning May 1, 2009, to August 1, 2010, using the search terms "influenza attack rate" and "influenza infection rate." The inclusion criterion was all English-language articles that provided infection rate estimates and explicitly described the methods used to derive the estimates.

Different methods for estimating infection rates

From the common methods used globally (5, 7–20) (Table 1), we selected 4 representative generic methods (Table 2), together with generic equations and minimum data requirements, to determine their comparability. The 4 methods were serologic cohort and cross-sectional studies, sentinel general practitioner (GP) ILI surveillance, and laboratory surveillance to supplement GP data.

Using data from the first wave of the 2009 H1N1 epidemic in Singapore, a tropical city-state, we compared infection rates estimated using these methods. Singapore was ideal for this study, because the first epidemic wave's temporal progression was well-defined—beginning in late June, peaking in early August, and ending by September (Figure 1)—and several surveillance programs and studies were performed simultaneously in the adult population, facilitating comparison of different methods.

Data sources

We used data sources available from June 2009, at the first suggestion of community transmission in Singapore, to October 2009, 1 month after numbers of respiratory illness cases returned to baseline levels (Figure 1). ILI cases were defined as cases involving new-onset respiratory symptoms with body temperature greater than 38.0°C (100.4°F), following World Health Organization definitions (21, 22). We performed aggregated and age-stratified analyses among 5 age groups: 20–24, 25–34, 35–44, 45–54, and ≥55 years. Data sources included:

1) A paired seroincidence adult cohort study (17). Multiple blood samples were obtained from each participant, including a baseline sample taken up to June 27, 2009 (before the local epidemic); a second sample taken between August 20, 2009, and August 29, 2009 (4 weeks after the epidemic's peak); and a postepidemic sample taken between October 6, 2009, and October 11, 2009 (4 weeks after the epidemic subsided). Fortnightly telephone surveys were used to collect data on clinical symptoms and health-care consultations. Data from 727 participants with paired serum samples were used.
2) A sentinel GP network of 23 GPs nationwide reporting ILI cases, initiated in June 2009 (23). Individual patient consultations involving ILI were recorded using a standardized template and submitted daily, together with basic demographic details.
3) Laboratory-based national surveillance by the Ministry of Health using samples from ILI patients visiting sentinel primary health-care clinics. Samples were tested for 2009 influenza A virus (H1N1) by means of reverse-transcriptase polymerase chain reaction (RT-PCR) (24), producing weekly age-stratified data on the proportion of ILI samples positive for H1N1-2009. When combined with ILI surveillance data, this negates the need to estimate the proportion of ILI consultations due to influenza (ILI consultations include conditions not due to influenza), leaving only the proportion of influenza cases who seek medical consultation for ILI to be determined.

Data from the serologic and GP studies were collected under the approval of the National University of Singapore Institutional Review Board. Laboratory data were part of the Ministry of Health's ongoing influenza epidemiology surveillance program, and no ethics review was required.

Statistical methods and computation of infection rates

In addition to the main data, each method required supplementary data ranging from simple test sensitivity for paired serologic data to consultation rates given infection and infection rates given ILI consultation. While serologic surveys intrinsically account for asymptomatic infections, ILI-based estimates need to be complemented with prior information to allow for nonreporting of symptomatic cases and asymptomatic infections. Because the latter information was available from the serologic surveys, we used parameters derived from the serologic surveys for the ILI-based estimates.

To allow full propagation of parametric uncertainty, we used an objective Bayesian approach, taking flat prior distributions in the absence of data and informative priors only when suitable external data were available. We used as many data as were available from these studies, including some which would not be available in other settings using only 1 source of data. Full details on the statistical methods used and the distributions of key parameters can be found in the Web Appendix (http://aje.oxfordjournals.org/).

Method 1: paired serologic surveys. To estimate infection rates from paired serologic surveys, we defined overall seroconversion as a 4-fold or greater rise in titer on hemagglutination inhibition (HAI) testing between baseline titers and subsequent samples for the same individual. Since not all influenza infections may be detected by HAI (because of sample timing, insufficient titer increases, or measurement error), we adjusted the seroconversion rates by HAI sensitivity using data from our study and another study (17, 25). Because there was no clear evidence on HAI false-positive rates, we did not adjust for this possibility.

Method 2: cross-sectional serologic surveys. To estimate infection rates from cross-sectional sampling similarly to

other studies, we defined the cross-sectional seroprevalence at each sampling point as the proportion with HAI titers of $\geq$40 (12, 14). We then subtracted baseline seroprevalence from final seroprevalence and adjusted the results by the sensitivity of a single postinfection sample to detect HAI titers of $\geq$40 in patients confirmed to have infection.

Method 3: ILI data from sentinel GPs. When using ILI data from sentinel GP sites, we computed the number of ILI consultations per sentinel GP day and scaled this to the population using the relative proportion of ILI seen by the average GP, using data on primary-care consultations from a national survey (26). In addition, we estimated the ratio of all ILI consultations to influenza infections through data on symptoms and health-care-seeking behavior available from our serologic cohort study (adjusting for HAI sensitivity), assuming that the serologic study was representative of the general population in terms of symptom presentation and health-care-seeking behavior. We then estimated the number of community influenza infections given the ILI observed. In the absence of such data, other approaches must be taken to scale the estimates from the sampled data to the general population appropriately (see Discussion).

Method 4: laboratory surveillance and ILI data from sentinel GPs. We also used laboratory data to supplement sentinel GP ILI data, replacing the proportion of ILI consultations due to influenza with the proportion of ILI samples that tested positive for H1N1-2009 by RT-PCR, while adjusting for the imperfect sensitivity of the RT-PCR assay in detecting influenza cases (25). Ideally, validation should be performed in the same laboratory using the same virus strain and correlated with epidemiologic data; because this was not possible, we performed sensitivity analysis to account for it. We then incorporated the fraction of infections without a primary care consultation for ILI from our cohort study as above.

Because of the poor specificity of acute respiratory illness in estimating influenza (27), we did not include analysis relying on acute respiratory illness only.

Sensitivity analyses

Because not all countries have access to relevant supporting data, especially on ILI consultation rates, some methods require extrapolation from other settings. Therefore, we performed Bayesian sensitivity analyses to determine the robustness of these methods to misspecification of key input parameters and the resulting impact on inferred infection rates. We set Dirac delta priors on one parameter at a time, keeping all other priors as above and varying the single parameter in question over a plausible range, as might be done operationally when no accurate data are available. The parameters examined were the sensitivity of the tests, the ratio of all ILI consultations to influenza cases, and the market share of sentinel GPs—factors that may vary by strain, location, and time.

Analysis of sample-size effects

Finally, to appreciate the effect of sample size on the spread of estimates for future surveys, we performed bootstrap analysis on our existing data. For methods 1 and 2, we simulated, using a binomial distribution, the proportion of infections which might be observed to seroconvert with different numbers of paired sera or to have antibodies at titers $\geq$40 in different numbers of baseline and follow-up samples, respectively, assuming that the true infection rate corresponded to our estimate. For methods 3 and 4, we simulated the observations for a situation in which the sentinel GP ILI data had been derived from different numbers of GPs, by resampling with replacement from the available GPs (we restricted resampling to GPs who submitted data for at least 50% of all days). To estimate the effect of laboratory samples on method 4, we used the binomial distribution to simulate positive proportions which might be observed in each week, assuming that laboratory samples were distributed uniformly each week across the epidemic. The corresponding formulae were applied to the estimates derived from the bootstrap with 100,000 resamples for each method and sample size. Since the availability of external data in future outbreaks is unpredictable, we did not attempt to incorporate parametric uncertainty from external data in these analyses. We used a 5- to 10-percentage-point spread in the 95% confidence interval of the infection rate estimate as reasonable for classifying epidemic severity or for evaluating the success of interventions.

RESULTS

Figure 2 shows the estimated infection rates calculated from the various estimation methods based on the Singapore studies. The overall infection rate estimated using paired seroconversion samples was 17% (95% Bayesian credible interval (BCI): 14, 20). Using estimates derived from paired seroconversion data as the comparison group, the overall estimate derived from cross-sectional serologic sampling (obtained with baseline and final titers from the serologic cohort study as independent samples) was lower at 12% (95% BCI: 9, 17), also observed across all age groups. Estimates from ILI rates (15%, 95% BCI: 10, 25) and estimates from the combination of ILI and laboratory data (12%, 95% BCI: 8, 18) provided overall estimates close to the serologic estimates, although there were variations among various age groups.

The substantial overlap in 95% Bayesian credible intervals for all 4 methods, along with fairly close point estimates, suggests that accurate determination of input variables can produce similar results regardless of the estimation method. The actual and effective sample sizes available to us led to estimates from ILI alone being the most uncertain, while seroconversion data gave the most precise estimates, although this may have been different if resources had allowed for different relative sample sizes. Estimates using the combination of ILI and laboratory data were less sensitive than ILI alone but more sensitive than the seroconversion estimates.

From the sensitivity analyses (Web Figure 1), serologic cohort estimates were very robust to misspecification of the external input parameter (test sensitivity), as were cross-sectional serologic estimates. The latter, however,

Table 1. Results From Studies That Estimated Infection Rates for H1N1 Influenza A, 2009

First Author, Year (Reference No.)	Study Location	Study Period	Estimated Infection Rate	Method of Estimation	Details
Lipsitch, 2009 (7)	Mexico	April 2009	0.11%–0.35% during the month of April 2009 (population of 106,682,518)	Surveillance data from travelers	International public health records surveyed to estimate infection rates among travelers to Mexico Cases among Mexican residents = cases in travelers × (Mexican population × 30 days)/(traveler population × duration of travel)
D'Ortenzio, 2010 (8)	Réunion Island, France	May 2009–September 2009	12.85% (104,067/810,000)	Sentinel physician network, cross-sectional ARI prevalence survey	Incidence of ARI consultations gathered from social insurance data, adjusted by the proportion of sentinel physician consultations Health-care-seeking behavior in persons with ARI from a cross-sectional survey Calculated by extrapolating the proportion of randomly selected ARI patients testing H1N1-positive in the total estimated no. of ARI cases
Dawood, 2010 (9)	Hunter New England, Australia	June 1, 2009–August 30, 2009	6.2% (range, 4.4%–8.2%) 53,383 (range, 37,828–70,597) out a population of 866,565	Syndromic surveillance and laboratory data	Incidence of ILI from an online self-reporting ILI surveillance system Proportion of ILI samples that tested H1N1-positive from national laboratories Using these data, the proportion of ILI cases due to H1N1 was estimated and extrapolated to the general population.
Gordon, 2010 (10)	Nicaragua	June 1, 2009–November 15, 2009	20.1% among children aged 2–14 years	Syndromic surveillance, laboratory testing	Cohort of children selected from an existing dengue study Testing criteria were fever with cough, sore throat, or rhinorrhea Samples were tested by RT-PCR to determine the H1N1 clinical attack rate. No extrapolation to the general population was done.
Flahault, 2009 (5)	France	September 2009–December 2009	10.6% among pregnant women 1,712,000 cases (95% CI: 1,112,700, 2,311,300) in persons aged 20–39 years	Cross-sectional seroprevalence	Cross-sectional seroprevalence study from serum obtained from pregnant women in weeks 48–49 of 2009 Cumulative seroprevalence was then estimated for the population aged 20–39 years.
Moghadami, 2010 (11)	Iran	December 2009	58.9% (1,504/2,553)	Cross-sectional seroprevalence	Single-sample cross-sectional seroprevalence study Serum samples from randomly selected participants in the community
Miller, 2010 (12)	England, United Kingdom	August 2009–September 2009	Age group, years <5: 21.3% (95% CI: 8.8, 40.3) 5–14: 42.0% (95% CI: 26.3, 58.2) 15–24: 20.6% (95% CI: 1.6, 42.4) 25–44: 6.2% (95% CI: −2.8, 18.7) 45–64: −2.7% (95% CI: −10.3, 7.1) ≥65: 0.9% (95% CI: −8.8, 13.3)	Cross-sectional seroprevalence	Cross-sectional seroprevalence study involving pre- and postpandemic samples from blood collected for other purposes Infection rates were estimated by subtracting prepandemic seroprevalence from postpandemic seroprevalence.

Table continues

Table 1. Continued

First Author, Year (Reference No.)	Study Location	Study Period	Estimated Infection Rate	Method of Estimation	Details
Chan, 2010 (13)	Taiwan, Republic of China	October 2009–November 2009	30.8% among health-care workers 12.6% among controls	Cross-sectional seroprevalence	Single-sample cross-sectional seroprevalence study Serum samples taken from hospital staff and controls
Ross, 2010 (14)	Pittsburg, Pennsylvania, United States	Mid-November–early December 2009	21% (unadjusted) Range from 5% for persons aged 70–79 years to 45% for persons aged 10–19 years Baseline 6% among young adults aged 18–24 years	Cross-sectional seroprevalence	Cross-sectional seroprevalence study with pre- and postpandemic samples Prepandemic samples only from young adults aged 18–24 years Postpandemic samples from laboratory specimens collected for other purposes over a wide age range
Allwinn, 2010 (15)	Germany	November 2009	12% (27/225) with titer of ≥1:40 (unadjusted) Baseline 13.1% (19/145) with titers of 1:>32	Cross-sectional seroprevalence	First sample from blood donors previously recruited for a serum survey of the spread of enterovirus 71 infection Second sample from randomly selected patients at a local university hospital
Grills, 2010 (16)	Australia	August 2009–October 2009	10% in adults aged 18–65 years	Cross-sectional seroprevalence	Participants in a health monitoring program were tested opportunistically. Baseline prepandemic seropositive rate from another study was subtracted from the result.
Chen, 2010 (17)	Singapore	June 22, 2009–October 15, 2009	13.5% in community-dwelling adults 6.5% in hospital staff 29.4% in military personnel 1.2% in long-term-care patients	Serologic cohort study	Multisample seroepidemiologic cohort study Serial serum samples from individuals Seroconversion was determined by a 4-fold rise in titers.
Crum-Cianflone, 2009 (18)	San Diego, California, United States	April 21, 2009–May 8, 2009	0.53% (101 per 100,000) from April 21, 2009, to May 8, 2009	Complete testing of ILI cases	Complete RT-PCR testing of all ILI cases from a captive population of local US military beneficiaries
Colizza, 2009 (19)	Mexico	April 2009	0.11%–1.31% (121,000–1,394,000 cases as of April 30, 2009)	Mathematical modeling	Model with a geographically structured metapopulation approach Use of a population-level census, human mobility flows, and disease dynamics to model disease evolution and infections
Presanis, 2009 (20)	Milwaukee, Wisconsin, and New York, New York, United States	April 2009–July 2009	Not shown; used as a denominator to determine hospitalization and case-fatality rates	Mathematical modeling Data from physician consultations, laboratory, and telephone survey	Estimation using mathematical model and probabilities of ILI with consultations, consultations that were tested, and proportion positive. For New York, a telephone survey was conducted to determine self-reported ILI status.

Abbreviations: ARI, acute respiratory illness; CI, confidence interval; ILI, influenzalike illness; RT-PCR, reverse-transcriptase polymerase chain reaction.

were strongly influenced by misspecification of the level of baseline prepandemic titers. Because substantial proportions of persons had baseline antibodies to H1N1-2009 (12, 17, 28), accurately determining baseline rates is important, and cross-sectional estimates that assumed no baseline titers (similar to the 0% baseline value in Web Figure 1C) would bias infection rate estimates upwards.

ILI estimates were very sensitive and changed substantially with key parameters of market share per GP, proportion of influenza cases who seek medical consultation for ILI, and proportion of ILI consultations due to influenza. Estimates derived from combining ILI data with laboratory data only required determining the proportion of influenza cases that sought medical consultation for ILI, which we

Table 2. Methods Used for Estimating Rates of Influenza Infection During the 2009 H1N1 Outbreak in Singapore

Method and Data Requirements	Advantages (+) and Disadvantages (−)
Method 1: paired serologic surveys[a]	
Seroconversion data from cohort study	+ Detects subclinical cases
Sensitivity of the serologic test to detect true infection	− Difficulties in timely data collection during an evolving pandemic
Total population size (to determine confidence interval for the estimate)	− No estimate of clinical infection rate
	− Availability of results is dependent on sampling intervals
Method 2: cross-sectional serologic surveys[b]	
Proportion of persons with high pre- and postpandemic titers	+ Relative ease of data collection in comparison with paired serologic surveys
Sensitivity to detect change in titers (proportion of true infections that have high postpandemic and low prepandemic titers using the cutoff titer)	− Risk of underestimation because of persons with high baseline titers
Total population size (to determine confidence interval for the estimate)	− Difficult to generalize to population when using banked samples
Method 3: syndromic surveillance for ILI[c]	
Data on all ILI consultations from sentinel GPs	+ Allows for "real-time" estimation of infection rate
Proportion of influenza cases involving consultation for ILI	+ Data collection is possible with minimal resources
Proportion of ILI consultations due to influenza	− Unable to capture subclinical infections
Market share of GPs surveyed among the total population	− Dependent on clinician reporting
Total population size	− Difficulties in estimating input parameters
	− Large margin of error if given inaccurate data
Method 4: syndromic surveillance for ILI with virologic data[d]	
Data on all ILI consultations from sentinel GPs	+ Margin of error is reduced in comparison with method 3
Market share of GPs surveyed among the total population	+ Allows for "real-time" estimation of infection rate
Proportion of influenza cases involving consultation for ILI	− Additional resources required for laboratory testing
Laboratory proportion of ILI samples that test positive for influenza	− Dependent on sensitivity of laboratory test
Sensitivity of the laboratory test	
Total population size	

Abbreviations: GP, general practitioner; ILI, influenzalike illness.

[a] Method 1 infection rate = (no. of persons who seroconverted)/[(total no. followed up) × (sensitivity of the serologic test)].

[b] Method 2 infection rate = [(proportion with high postpandemic titers) − (proportion with high prepandemic titers)]/(sensitivity to detect true change in titers).

[c] Method 3 infection rate = (no. of ILI cases)/[(market share of GPs surveyed) × population × (proportion of influenza cases that involved consultation for ILI) × (proportion of ILI consultations due to influenza)].

[d] Method 4 infection rate = (no. of ILI cases)/[(market share of GPs surveyed) × population × (proportion of influenza cases that involved consultation for ILI) × (proportion of ILI samples that tested positive/sensitivity of the laboratory test)].

obtained from our serologic study questionnaire. Infection rate estimates were very sensitive to misspecification of this parameter; however, were it determined with greater accuracy, this method would provide extremely accurate estimates, as shown by the very narrow Bayesian credible intervals in Web Figure 1H.

Figure 3 shows the sample sizes required to obtain 5- and 10-percentage-point spreads in mean infection rates for the 95% confidence intervals. For paired serologic estimates, 300 participants were required in order to achieve a 10-percentage-point spread, and 1,150 were required for a 5-percentage-point spread. For cross-sectional serologic estimates, more persons are needed per survey to achieve a similar spread. For ILI estimates alone, the required number of GPs that report daily had to be 20 and 90 to achieve spreads of 10- and 5-percentage points, respectively (Singapore had an estimated 2,138 GPs in 2009) (26). Adding laboratory data reduces the number of GPs needed by almost half, while the total number of laboratory samples required over the entire study period was less than 200 for a 5-percentage-point spread.

Table 1 summarizes data on the 15 papers selected from our literature search, out of 295 identified. These studies did not all use the same methods, and it is difficult to compare their results because most investigators did not completely adjust for key parameters such as test sensitivity, asymptomatic cases, or baseline titers. Three studies used surveillance data—from travelers to Mexico early in the epidemic (7), from a sentinel physician network (8), and from online surveillance tools together with laboratory data (9); and all used different methods of scaling data to the population

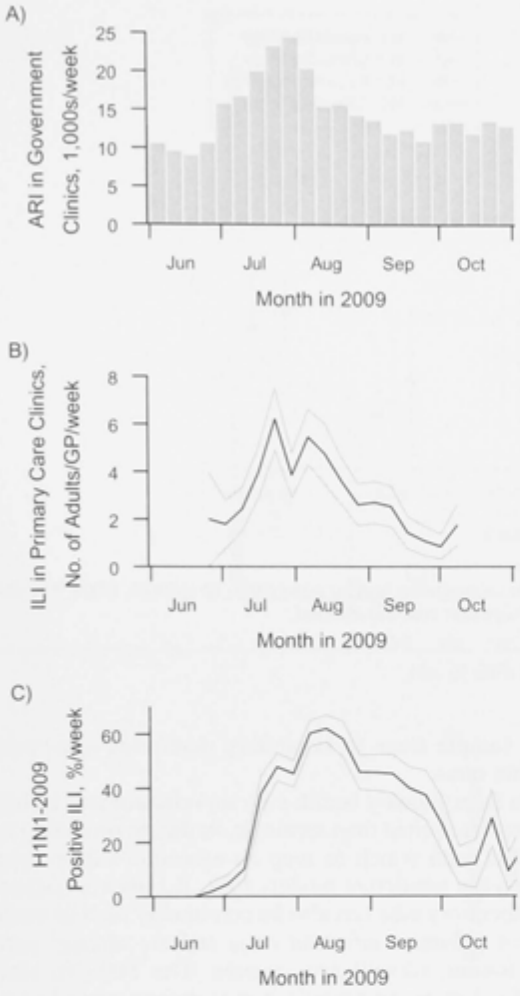

Figure 1. Sources of available data on influenza infection in Singapore from June to October 2009. A) Numbers of cases of acute respiratory illness (ARI) diagnosed in government clinics, in thousands per week; B) numbers of adult cases of influenza like illness (ILI) reported by primary-care general practitioner (GP) sentinel clinics per GP per week; C) percentage of ILI cases that tested positive for H1N1-2009 influenza per week. Lighter lines, 95% confidence interval.

level. Of the serologic surveys, only 1 study used paired samples (17); 7 studies were cross-sectional, with different sample origins (5, 11–16); and only 2 adjusted for prepandemic seroprevalence (12, 16). Two studies performed laboratory testing of all ILI cases but in unique small-scale military (18) and pediatric cohort (10) settings, while 2 used mathematical modeling of primary data (19, 20).

DISCUSSION

Estimation of epidemic infection rates is important in order to evaluate disease morbidity and to obtain accurate denominators for severity indicators, such as hospitalizations or case fatality. Attempts have been made to determine infection rates through different methods during different time periods (Table 1). However, none describe the relative comparability and robustness of these estimation methods in a single setting. Public health professionals and policymakers should understand the advantages and disadvantages of these methods to incorporate data collection into preparedness plans and to account for possible errors.

Serologic surveys provide reliable estimates of infection rates, since they determine antibodies even for asymptomatic cases (17, 29). Serial sampling from individuals in the context of H1N1-2009 is important because baseline antibodies were present from cross-reactivity to different strains (28). Serial sampling requires preplanning and good timing to establish cohorts with baseline blood samples before the epidemic's onset. Therefore, few countries have been able to perform serologic cohort studies (6). Serologic surveys are only available after each sampling interval, depending on laboratory capacity; will usually not provide real-time estimates; and are unable to detect temporary rises in titers that may arise from mild infections, which may be important for subsequent immunity. A further weakness of serologic surveys is that they do not estimate clinical infection rates unless clinical surveys are conducted simultaneously.

Cross-sectional serologic surveys have disadvantages similar to those of cohort studies but are easier to conduct without individual follow-up, and samples can be obtained from other collection sources (e.g., blood banks). However, upon subtraction of baseline prepandemic levels, they may produce lower estimates than cohort studies because of overcompensation for baseline titers (12, 17, 28). This may result in estimates with negative infection rates, which are difficult to interpret (12). This may also be a problem when producing age-stratified estimates if baseline antibody levels differ by age (12, 14, 28). Other surveys used only a single postpandemic sample without baseline adjustment (5, 11, 13–15), which may have resulted in overestimation; estimates were as high as 58.9% in one study (11) and were 21% in another study, which also had a 6% baseline prevalence of antibodies (14). Unless accurate baseline estimates are available, cross-sectional surveys will be less accurate than paired surveys. The sample source may also make it difficult to generalize results—some studies obtained blood collected for other purposes, including blood donations and health monitoring programs, which may not represent the general population (12, 14–16).

It is clear from our sensitivity analyses that serologic survey estimates result in narrower ranges and are less sensitive to misspecification of input parameters. However, how serologic titers decrease over time is unknown, especially if samples are taken at long intervals. This can be averted by conducting serologic cohort studies with multiple samplings at shorter intervals.

ILI-derived estimates are easily obtained from sentinel GPs, and in Singapore they were similar to serologic and laboratory estimates. However, ILI estimates are very sensitive to changes in input parameters, and these must be determined accurately. Adjustment for nonreporting and asymptomatic infection can be achieved via a "scaling-up

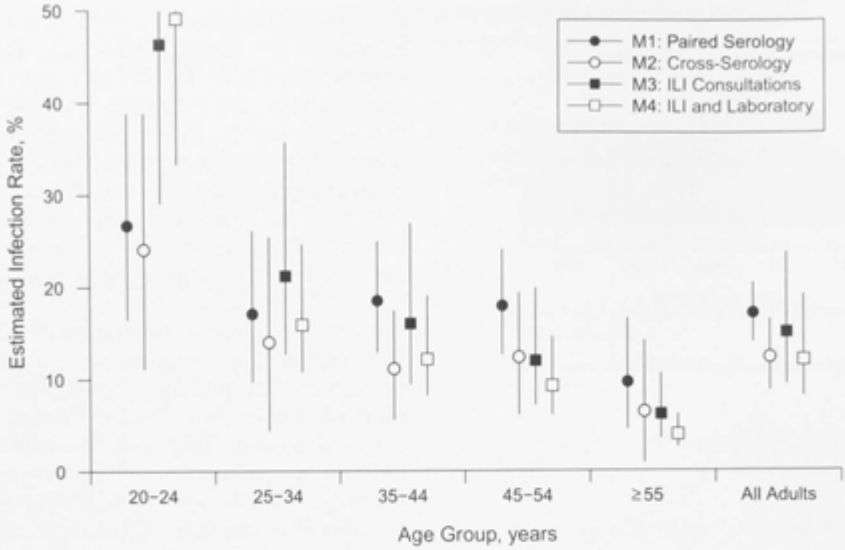

Figure 2. Rates of H1N1-2009 influenza infection estimated from various methods, aggregated and by age group, Singapore, 2009. For details on methods 1–4 (M1–M4), see Table 2. ILI, influenza like illness. Whiskers, 95% Bayesian credible interval.

factor" or by using prior information on ILI consultations for all influenza infections. The latter is intrinsically difficult to obtain, since it is ideally based on data from confirmed cases, which were available for our study (17). In other settings, strategies might involve extrapolating from past epidemics or other regions. For example, because different adjustment factors were used in the studies by D'Ortenzio et al. (8) and Dawood et al. (9), these estimates are unlikely to be comparable. Adding accurate laboratory testing data to ILI addresses the otherwise substantial difficulty in estimating ILI consultations due to influenza, and results in estimates that are less sensitive to parameter misspecification. This does not obviate the need to estimate the proportion of influenza cases who seek medical consultation for ILI, which we did via our serologic cohort (17), although this proportion can also be estimated through local surveys carried out among ILI cases (since consultation is influenced by local health-care-seeking behaviors) (2), adjusted by the proportion of ILI among influenza cases, which is a biologic variable that presumably can be extrapolated from other regions. The need for reliable extraneous data is the main weakness of consultation data, especially in heterogeneous environments.

Infection rates differ across age groups, with the highest infection rates being seen in younger adults, confirming that young adults (and perhaps children) had higher infection rates during the 2009 H1N1 pandemic. Estimates from different estimation methods also differ across age groups: Greater differences exist between estimates in the younger age groups, with ILI-derived estimates being biased upwards relative to serology (although the 95% Bayesian credible intervals overlap). This shows the difficulties in estimating attack rates for different age categories through surveillance, without having accurate scaling factors and concomitantly larger sample sizes to accurately determine age-specific infection rates.

Data from primary health-care surveillance and laboratories are more suited than serologic studies to providing real-time data with which to map an epidemic's development and develop predictive models (23). ILI-derived estimates with laboratory data can also be continually used to monitor seasonal influenza infection rates and are already part of many routine surveillance systems. The Mexican studies carried out at the epidemic's start to determine early extent of spread (7, 19) and the localized San Diego, California, outbreak (18) provided real-time estimates for early planning. However, additional laboratory data may not be readily available in low-resource settings and may be difficult to obtain in a heterogeneous setting with different sociodemographic profiles within a country.

Another potential obstacle to accurate estimation of infection rates is the sample size required for sufficient accuracy. Serologic studies required to achieve a 5-percentage-point spread ($\geq$1,000 participants per survey) may be difficult to perform in settings with fewer resources. ILI estimates may be easier to collect if GPs are able to routinely report ILI cases, since 4% of all GPs can achieve a 5-percentage-point spread. Including laboratory samples further reduces the number of GPs required, while only requiring a small number of samples over the epidemic period because of good correlation between influenza-positive laboratory samples and the epidemic curve. With a consistent sentinel GP network and laboratory testing program, method 4 can be routinely used to estimate infection rates for regular influenza seasons and the relative burden of disease from different strains.

Two limitations of our study were the lack of pediatric data for comparison (these data were collected differently

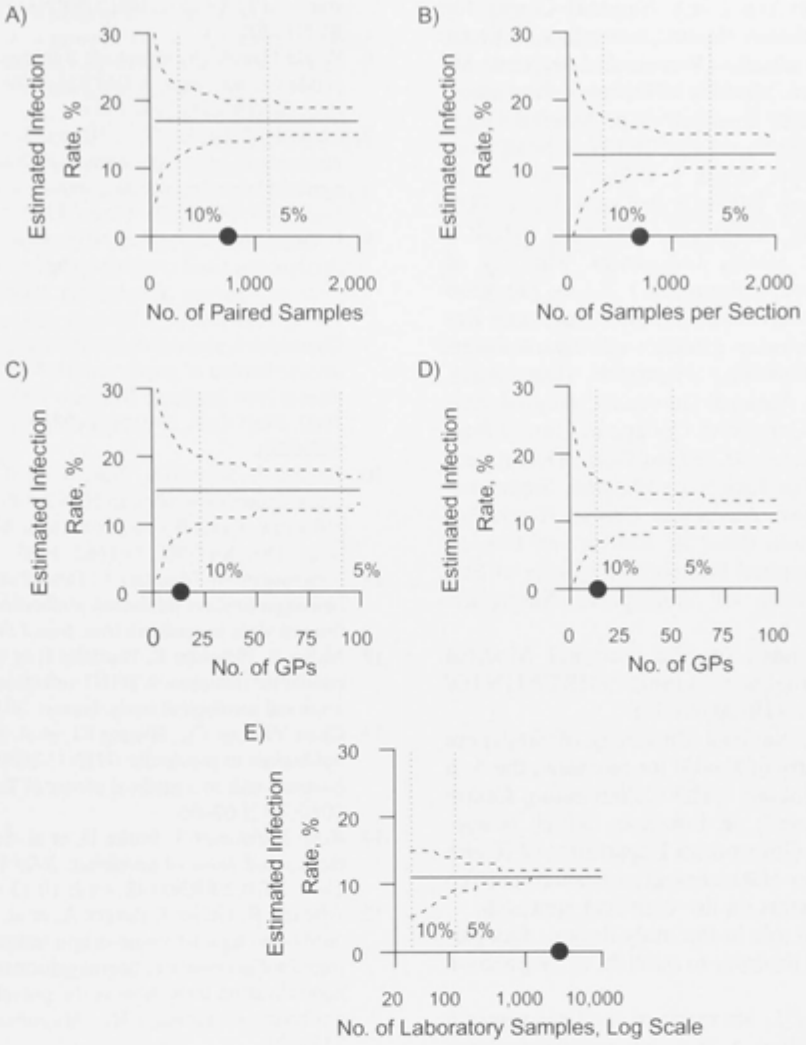

Figure 3. Change in the 95% confidence interval (dashed lines) for the mean estimated H1N1 influenza infection rate (solid line) with different sample sizes using 4 different estimation methods, Singapore, 2009. A) Method 1; B) method 2; C) method 3; D) method 4; E) method 4 (see Table 2). Sample sizes which resulted in a 5- and 10-percentage-point spreads in the confidence interval for the mean estimates are shown with dotted vertical lines; the actual sample size used in the Singapore studies is shown with a circle on the *x*-axis. The total number of general practitioners (GPs) in Singapore in 2009 was approximately 2,138.

from data on adults) and small sample sizes when stratifying by age for some analyses. Researchers who aim to estimate age-group-specific infection rates will need to increase the sample size proportionally, which could result in very large studies. In this paper, we have clearly displayed the differences between the methods in a single population, and these concepts are applicable to other populations and settings. Although this study was based on Singapore's H1N1-2009 epidemic, the methods proposed are applicable globally to other infectious diseases.

Estimates of infection rates from serologic data and ILI data with or without laboratory data can provide comparable results if input parameters are accurately determined. Each method has advantages and disadvantages which should be considered when comparing estimates. The epidemic timing, objectives of data collection, and availability of resources will also determine the method used. Countries with sufficient resources may consider using multiple estimation methods to cover the disadvantages of some while benefiting from the advantages of others.

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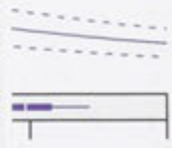
Web Figure 1. One-way sensitivity analyses showing changes in the estimated infection rates using various methods, with changes to single input parameters. Graphs A to H show changes to the infection rate estimates using the various methods (labeled M1 to M4, respectively) shown on the *y*-axis with changes to the input parameters shown on the *x*-axis. The mean and 95% Bayesian credible intervals of the sensitivity analyses are shown in the main graphs. The box plots or point estimates of the actual parameters obtained from the Singapore studies are shown below each graph, together with the box plots of the actual results on the left.

(b)



80 100
Sensitivity, %

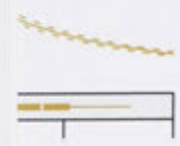
(d)

75 100
if ILI), %

(f)

20 30
if H1N1v), %

(h)



20 30
if H1N1v), %

Appendix to Comparability of different methods for estimating influenza infection rates over a single epidemic wave

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Statistical methodology

All four approaches to estimate infection rates require information from multiple sources. The Bayesian statistical paradigm (1) is particularly well suited to combining information from different sources while allowing for proper propagation of uncertainty. For all four methods, we use non-informative, flat prior distributions on all parameters unless external data were available to provide an informative prior distribution. For these informative prior distributions, we started with a non-informative prior, fit a model to the external data, and used the resulting posterior distribution *including all within model uncertainty* as the informative prior for the main analysis. Where an analytical form could not be found for it, the posterior distribution from fitting to external data was approximated by a (multivariate) Gaussian distribution.

When conjugate priors were not available, models were fit using Markov chain Monte Carlo integration, with univariate Gaussian proposal distributions centred on the current value and the Metropolis–Hastings acceptance algorithm (1). Proposal bandwidths were selected by trial and error until satisfactory mixing of MCMC chains was achieved; convergence of MCMC output was assessed by visual inspection of trace plots. When mixing was slow, chains were run for longer and thinned to reduce autocorrelations, but for models in which the MCMC routine mixed ostensibly quickly through the posterior distribution, no thinning was used.

Method 1: paired serological surveys

Method 1 used paired serology, with a baseline blood sample and the highest follow up sample, either mid- or post-epidemic. A "fourfold rise" (from baseline in (1:t,1:2t) to (1:4t,1:8t) for $t \in \{5, 10, 20, \ldots\}$; note that although this does not guarantee a fourfold rise in the uncensored titres standard terminology is to call it a fourfold rise, a convention we abide by here) is deemed to be a seroconversion. The proportion seroconverting must however be scaled up to account for imperfect sensitivity of serology. To do this, we used data on seroconversions associated with RT-PCR confirmed infection from (2) and (3).

Data used:

- number of seroconversions;

- number of possible seroconversions;
- estimated sensitivity of seroconversion.

The number of seroconversions and number at risk were taken from a seroepidemiological study performed in Singapore during the first wave (3). The sensitivity of seroconversion relative to RT-PCR confirmed infection was estimated from historic (2) and contemporary (3) studies. The estimates from these two studies individually were close enough to warrant merging the two and using the historic data alongside the 2009-H1N1 data.

Notation:

- n = the number of individuals in follow-up—known;
- x = the number of individuals that seroconverted—known;
- σ_1 = sensitivity of the seroconversion test versus RT-PCR, i.e. the probability of a seroconversion given a virologically confirmed infection;
- p = the proportion infected in the population.

Full model for data and parameters:

$$\begin{aligned} x &\sim \text{Bin}(n, \sigma_1 p) \\ \sigma_1 &\sim \text{Be}(675, 174) \\ p &\sim \text{U}(0, 1). \end{aligned}$$

The prior distribution for σ_1 comes from taking the conjugate prior $\text{U}(0,1) = \text{Be}(1,1)$ to a binomial model for seroconversion given RT-PCR confirmed infection using the data in (2) and (3).

Simplified representation of model:

$$\begin{aligned} \hat{p} &= \frac{x}{n\hat{\sigma}_1} \\ \hat{\sigma}_1 &= 0.79 \end{aligned}$$

Assumptions:

- The findings from (2) and (3) generalise to our study population. The two papers show similar results, supporting this assumption.
- Our cohort is a random sample from the population—the fact that it is not means there may be a systematic and unquantifiable bias in the results.
- Seroconversions are independent—some study participants had co-habitants also in the study, leading to a systematic underestimate of the uncertainty. The magnitude of the bias is likely to be small due to the small fraction of households with multiple members in the study.

Table 1: Posterior mean and 95% credible intervals for parameters of method 1

Parameter	Estimate	Lower bound	Upper bound
σ_1	0.79	0.77	0.82
p	0.17	0.14	0.20

Estimation:

The joint distribution of (σ_1, p) is sampled via Markov chain Monte Carlo.

Method 2: cross-sectional serological surveys

Method 2 uses cross-sectional serological samples of the population. We emulate this by delinking the paired serology data. In both samples, a titre of 1:40 is taken as evidence of exposure to the virus. To account for pre-existing antibodies (resulting from cross reaction to other strains or measurement error), the estimated proportion above this threshold pre-epidemic is "subtracted" from the estimated proportion post-epidemic.

Data used:

- number of people at pre- and post-epidemic with titres above 1:40;
- number of people in these two samples;
- cross sectional antibody data from participants with RT-PCR confirmed infection.

The emulated cross-sectional data are derived from ref (3), as is the sensitivity estimate.

Notation:

- n_1 = the number of individuals giving samples at baseline—known;
- n_2 = the number of individuals at final follow up—known;
- x_1 = the number of individuals with high (above 1:40) initial titres—known;
- x_2 = the number of individuals with high final titres—known;
- q = the proportion of the population with naturally high titres, pre-infection (presumed);
- σ_2 = the proportion of the population with high titres at final follow that were infected and had low initial titres;
- p = the proportion infected in the population.

Table 2: Posterior mean and 95% credible intervals for parameters of method 2

Parameter	Estimate	Lower bound	Upper bound
σ_2	0.79	0.67	0.88
q	0.03	0.02	0.04
p	0.12	0.09	0.17

Full model for data and parameters:

$$\begin{aligned} x_1 &\sim \text{Bin}(n_1, q) \\ x_2 &\sim \text{Bin}(n_2, q + (1-q)\sigma_2 p) \\ \sigma_2 &\sim \text{Be}(46, 12) \\ q &\sim \text{Be}(3, 55) \\ p &\sim \text{U}(0, 1). \end{aligned}$$

The prior distributions for q and σ_2 come from taking the conjugate priors $\text{U}(0,1) = \text{Be}(1,1)$ to a binomial model for high titres at baseline and follow up for individuals with RT-PCR confirmed infection during the study using the data from (3).

Simplified representation of model:

$$\begin{aligned} \hat{p} &= \frac{x_2/n_2 - x_1/n_1}{(1 - x_1/n_1)\hat{\sigma}_2} \\ \hat{\sigma}_2 &= 0.79. \end{aligned}$$

Assumptions:

- The sensitivity and initial high titres findings generalise to our study population.
- Our cohort is a random sample from the population—which, again, it is not.
- Titre levels are independent (i.e. no household effects).
- We treat these as two independent cross-sectional surveys, but in actuality they are paired (see method 1 for the method accounting for pairing).

Estimation:

The joint distribution of (σ_2, q, p) is sampled via Markov chain Monte Carlo.

Method 3: ILI data from sentinel GPs

Data used:

- daily number of ILI consults at GP sentinel network;
- daily number of GPs reporting;

- interview of participants in serological study and their seroconversion status to estimate consultation rates;
- sensitivity of seroconversion (as method 1).

ILI consult data come from a GP sentinel network previously described (4). The questionnaire data are previously unpublished and were collected from the same group of patients as provided sera in ref (3). Sensitivity data sources are as in method 1.

Notation:

Unobserved:

- p = the proportion of the population infected;
- p_1 = the proportion of infections leading to ILI consults;
- p_2 = the proportion of ILI consults that correspond to actual infection;
- σ_3 = the probability of seroconverting given (RT-PCR confirmed) infection;
- N^s_{IVS} = the number of people infected, visiting primary care with an ILI and seroconverting in the serology–questionnaire study;
- N^s_{IS} = the number of people infected and seroconverting in the serology–questionnaire study;
- N^s_I = the number of people infected in the serology–questionnaire study;
- N^p_V = the number of people visiting primary care with an ILI in the population as a whole;
- κ = a parameter controlling the variability of GP consultations around the mean.

Observed:

- N^s_S = the number of people seroconverting in the serology–questionnaire study;
- N^s_{VS} = the number of people seroconverting and visiting primary care with an ILI in the serology–questionnaire study;
- N^s_V = the number of people visiting primary care with an ILI in the serology–questionnaire study;
- $\pi = 1/2486$ the proportion of primary care consultations attributable to a single GP in our study—assumed known;
- N^s = the number of people in the serology–questionnaire study;
- N^p = the number of people in the population (we restrict attention to resident adults throughout);
- D_t = the daily number of ILIs reported on day t;
- F_t = the number of GPs faxing or emailing a report on day t;
- $\mathcal{T}$ = the length of time of the two studies.

Full model for data and parameters:

$$
\begin{aligned}
N^s_{IS} &\sim \text{Bin}(N^s_I, \sigma_3) \\
\sigma_3 &\sim \text{Be}(675, 174) \\
(N^s_S - N^s_{VS}) &\sim \text{Bin}(N^s_{IS} - N^s_{IVS}, \sigma_3) \\
N^s_{IVS} &\sim \text{Bin}(N^s_{VS}, \sigma_3) \\
N^s_{IVS} &\sim \text{Bin}(N^s_{IS}, p_1) \\
N^s_{IVS} &\sim \text{Bin}(N^s_V, p_2) \\
N^s_I &\sim \text{U}(0, N^s) \\
D_t &\sim \text{NegBin}(\mu_t = F_t \pi N^p_V / \mathcal{T}, k = \kappa) \\
\kappa &\sim \text{U}(0, 10) \\
p_1 &\sim \text{U}(0, 1) \\
p_2 &\sim \text{U}(0, 1) \\
p &\sim \text{U}(0, 1) \\
N^p_V &= p p_1 N^p / p_2
\end{aligned}
$$

Simplified representation of model:

$$
\begin{aligned}
\hat{p} &= \frac{\hat{N^p_V} \hat{p_2}}{N^p \hat{p_1}} \\
\hat{p_1} &= \frac{N^s_{VS}}{N^s_S} \\
\hat{p_2} &= \frac{N^s_{VS}}{N^s_V \hat{\sigma}_3} \\
\hat{N^p_V} &= \frac{\sum_t \frac{D_t}{F_t}}{\pi}.
\end{aligned}
$$

Note that alternative simplified point estimates of the number of ILI consults in the population exist and it is not clear which gives the least bad representation of the estimate accounting for all within model uncertainty.

Assumptions:

- The sensitivity findings generalise to our study population.
- Our cohort is a random sample from the population.
- Seroconversions are independent (i.e. no household effects).
- The GPs in the surveillance network are representative.
- Observed ILIs in the GP study are negative binomial distributed, i.e. inflated by a factor κ relative to a Poisson to account for the inhomogeneous pattern of consults caused by the epidemic wave and day of week effects.

Table 3: Posterior mean and 95% credible intervals for parameters of method 3

Parameter	Estimate	Lower bound	Upper bound
σ_3	0.79	0.77	0.82
p_1	0.20	0.13	0.28
p_2	0.67	0.47	0.86
N^s_{IS}	124	112	137
N^s_{IVS}	24	20	30
κ	2.95	1.88	4.53
N^p_V	116 000	100 000	133 000
p	0.15	0.10	0.25

Estimation:

A two-stage approach is taken. In the first round, Markov chain Monte Carlo is used to sample the joint distribution of $(p_1, p_2, \sigma_3, N^s_{IVS}, N^s_{IS})$. A bivariate Normal distribution is then used to approximate the joint distribution of (p_1, p_2) which is then used as an informative prior for round two. In the second round, Markov chain Monte Carlo is used to sample the joint distribution of the population level estimands $(p_1, p_2, \kappa, N^p_V)$.

Method 4: Laboritory surveillance and ILI data from sentinel GPs

Here, we replace the estimated probability of H1N1 given ILI consult from the questionnaire associated with the serology study by an estimate from lab surveillance.

Data used:

- daily number of ILI consults at GP sentinel network;
- daily number of GPs reporting;
- weekly numbers of swabs of patients presenting with ILI testing positive and negative to H1N1;
- sensitivity of RT-PCR from (2);
- sensitivity of seroconversion (as method 1);
- interview of participants in serological study and their seroconversion status to estimate consultation rates.

Data sources as per method 3, with in addition laboratory data from Singapore's National Laboratory (published in part in ref (5)).

Notation:

Unobserved:

- L_w^I = the number of people sampled for lab testing that were infected in week w;
- p_1 = the proportion of infections leading to ILI consults;
- p_2 = the proportion of ILI consults that correspond to actual infection aggregated over the study period of the sero-cohort questionnaire, subsequently replaced by lab-derived estimates;
- p_{2w} = the proportion of ILI consults that correspond to actual infection in week w;
- σ^{PCR} = the probability of RT-PCR confirmed infection given *any* form of confirmed infection;
- σ^{sero} = the probability of seroconverting given (RT-PCR confirmed) infection;
- N_{IVS}^s = the number of people infected, visiting primary care with an ILI and seroconverting in the serology–questionnaire study;
- N_{IS}^s = the number of people infected and seroconverting in the serology–questionnaire study;
- N_{Vt}^p = the number of people visiting primary care with an ILI in the population on day t;
- N_{IVt}^p = the number of infected people visiting primary care with an ILI in the population on day t;
- N_{IV}^p = the number of infected people visiting primary care with an ILI in the population over the course of the study;
- N_I^p = the number of people infected in the population.

Observed:

- L_w^+ = the number of people sampled for lab testing that test positive in week w;
- L_w^- = the number of people sampled for lab testing that test negative in week w;
- N_S^s = the number of people seroconverting in the serology–questionnaire study;
- N_{VS}^s = the number of people seroconverting and visiting primary care with an ILI in the serology–questionnaire study;
- $\pi = 1/2486$ the proportion of primary care consultations attributable to a single GP in our study—assumed known;
- N^p = the number of people in the population (we restrict attention to resident adults throughout);
- D_t = the daily number of ILIs reported on day t;
- F_t = the number of GPs faxing or emailing a report on day t;
- $w(t)$ = the week that contains day t.

Full model for data and parameters:

$$
\begin{aligned}
L_w^+ &\sim \text{Bin}(L_w^I, \sigma^{\text{PCR}}) \\
L_w^I &\sim \text{Bin}(L_w^+ + L_w^-, p_{2w}) \\
\sigma^{\text{PCR}} &\sim \text{Be}(731, 62) \\
N_{IS}^s &\sim \text{Bin}(N_I^s, \sigma^{\text{sero}}) \\
(N_S^s - N_{VS}^s) &\sim \text{Bin}(N_{IS}^s - N_{IVS}^s, \sigma^{\text{sero}}) \\
N_{IVS}^s &\sim \text{Bin}(N_{VS}^s, \sigma^{\text{sero}}) \\
N_{IVS}^s &\sim \text{Bin}(N_{IS}^s, p_1) \\
N_{IVS}^s &\sim \text{Bin}(N_V^s, p_2) \\
p_1 &\sim \text{U}(0, 1) \\
p_2 &\sim \text{U}(0, 1) \\
\sigma^{\text{sero}} &\sim \text{Be}(675, 174) \\
p_{2w} &\sim \text{U}(0, 1) \\
N_{Vt}^p &\sim \text{Ga}(1, 1/100\,000) \\
D_t &\sim \text{Po}(F_t \pi N_{Vt}^p) \\
N_{IVt}^p &\sim \text{Bin}(N_{Vt}^p, p_{2w(t)}) \\
N_{IV}^p &= \sum_t N_{IVt}^p \\
N_I^p &= \frac{N_{IV}^p}{p_1} \\
p &= \frac{N_I^p}{N^p}
\end{aligned}
$$

Simplified representation of model:

$$
\begin{aligned}
\hat{p} &= \frac{\hat{N}_I^p}{N^p} \\
\hat{N}_I^p &= \frac{\hat{N}_{IV}^p}{\hat{p}_1} \\
\hat{p}_1 &= \frac{N_{VS}^s}{N_S^s} \\
\hat{p}_2 &= \frac{N_{VS}^s}{N_V^s \hat{\sigma}^{\text{sero}}} \\
\hat{N}_{IV}^p &= \sum_t \hat{N}_{IVt}^p \\
\hat{N}_{IVt}^p &= \frac{\hat{N}_{Vt}^p}{\hat{p_{2w(t)}}} \\
\hat{p_{2w}} &= \frac{1}{\hat{\sigma}^{\text{PCR}}} \frac{L_w^+}{L_w^+ + L_w^-} \\
\hat{\sigma}^{\text{PCR}} &= 0.92 \\
\hat{\sigma}^{\text{sero}} &= 0.79 \\
\hat{N}_{Vt}^p &= \frac{D_t}{F_t \pi}
\end{aligned}
$$

Assumptions:

- The sensitivity findings generalise to the lab samples.
- Our cohort is a random sample from the population.
- The GPs in the surveillance network are representative.
- Observed ILIs in the GP study an any particular day given the number of ILIs in the community on that day are Poisson.
- Lab samples were randomly drawn from the population of ILI cases.
- Independence of lab samples, seroconversions, infection status of patients consulting for ILI between and within days.
- The proportion of ILIs with H1N1 infection is piece-wise constant and changes only on Sundays with the beginning of a new e-week.

Estimation:

A three stage procedure is used. In the first round, the joint distribution of $(p_{2w}, \sigma^{\text{PCR}})$ is sampled using MCMC and the lab data. In the second round, the joint distribution of $(p_1, p_2, \sigma^{\text{sero}}, N_I^s, N_{IV}^s)$ is sampled (as in method 3) using Markov chain Monte Carlo. In the final round, the posterior distribution for all other estimands is sampled by Monte Carlo simulation, exploiting conjugacy to obtain beta posteriors for p_1 and gamma posteriors for N_{Vt}^p. The posterior distributions for all other terms can be sampled directly.

Table 4: Posterior mean and 95% credible intervals for parameters of method 4. Estimands that vary with time are not shown.

Parameter	Estimate	Lower bound	Upper bound
σ^{PCR}	0.92	0.90	0.94
σ^{sero}	0.79	0.77	0.82
p_1	0.20	0.13	0.28
p_2	0.67	0.47	0.86
N^s_{IV}	24	20	31
N^s_I	124	113	138
N^p_{IV}	61 000	57 000	64 000
N^p_I	317 000	216 000	499 000
p	0.12	0.08	0.18

Age stratified analyses

We undertook analyses of the infection rate in two ways:

- **age stratified**, in which infection rates in five age groups (20–24, 25–34, 35–44, 45–54 and 55 or over) were analysed independently. For methods using GP ILI consults, we assumed all ages had the same probability of consulting with an ILI when infected and that the proportion of ILIs due to H1N1 infection were the same for all ages. This assumption was needed since there was insufficient information on these proportions when the serological questionnaire was divided by age groups, as the number of ILI cases among seroconvertors within any particular age group was too small to obtain usable estimates. All other parameters were estimated using data from the subset of the data corresponding to that age group only.
- **non-age stratified**, in which we derived estimates of the adult infection rate under the assumption that infection rates are constant for all age groups. The motivation for this assumption is that for some of the age groups, there is considerable uncertainty on the model parameters, which can only be reduced by pooling information. An obvious, though complicated and computer-intensive, alternative to this homogeneity assumption that would also provide narrower estimates would be to develop an hierarchical model (see e.g. (1)) or to introduce a functional form for the effect of age on infection rates as part of a regression analysis.

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Chapter Five

Seroconversion to 2009 H1N1 Influenza in Singapore

In the previous chapter, we have shown that given the availability of good data, different methods of estimating influenza infection rates can yield overall estimates of similar magnitude. Of these, serological surveys are a good method to determine infection rates due to their ability to estimate probable infections that are sub-clinical or asymptomatic without the need to determine the expansion factors to account for these cases using traditional surveillance methods. Seroepidemiological studies have been proposed by the WHO as part of initial pandemic surveillance to understand the virulence, severity and clinical manifestation, as well as the effectiveness of various interventions (1-3). As such, the next study aims to determine the probable infection rates in different cohorts in Singapore, and the risk factors associated with seroconversion. I am the second author of the study (which is a large collection of subjects from four cohorts), and was the original study designer together with Dr Chen, the lead author. I led the military cohort section of this study as principal investigator.

To determine the seroconversion to 2009 H1N1 influenza in Singapore, we selected four different cohorts for the study for the following reasons:

1) The first cohort was taken from an existing serological cohort for chronic disease that was chosen to provide representation of the Singapore adult general population. This would provide the overall estimate of the infection rate in the adult population, and allows for comparison of the other groups.

2) The second cohort was from the military, a young population living in a semi-closed environment which will be the subject of most of the subsequent chapters. This is a unique cohort of individuals in Singapore due to the conscript military service for all male citizens and permanent residents for a

3) The third cohort was from hospital workers in a major public hospital in Singapore. Hospitals may be an area of increased transmission due to the interaction with infected individuals seeking treatment. At the same time, the use of personal protective equipment may provide protection to reduce transmission in the work environment, while the possibly better infection control training of hospital workers may reduce their risk of infection both in and out of the workplace.

4) The fourth cohort was residents in long-term care facilities that may be at risk of increased transmission due to the closed living environments, but this group comprised of a substantial proportion of older adults. In the context of the 2009 H1N1 pandemic, older adults were thought to have possible protective antibodies to the virus strain where up to a third of the elderly (≥65 years of age) had cross-reactive antibodies to the 2009 H1N1 virus prior to the onset of the pandemic (4).

Within each cohort, there are different sub-groups with possible reasons for different infection rates that are the subject of other manuscripts. One is the subject of Chapter Twelve and discussed in further detail there. Another study on the hospital

cohort showed that significant risk factors included being a nurse, and working in 2009 H1N1 influenza isolation wards. The study was published in Emerging Infectious Diseases in 2010 with myself as the second author, but not included in the thesis (5).

For this study, the laboratory test that was used was the HAI test. The other option was the virus micro-neutralization assay. A separate study in Singapore showed that HAI was comparable to the micro-neutralization assay (6). Although the latter test had slightly better sensitivity, HAI is much easier to perform in a timely manner. This is one of the key determinants of test selection, as the data would be needed to guide prompt public health policy decision making. That study also showed that increases in antibody levels that are detectable by HAI occur after two weeks post infection (6). In our study, we sampled the participants three to four weeks after the peak and end of the epidemic wave respectively to allow for adequate detection by the HAI test.

While the pediatric sub-population is an important group that needs to be considered in a population-wide analysis, it was unfortunately not possible to obtain a similar pediatric sample before the start of the local epidemic due to challenges obtaining the requisite approvals. This study therefore focuses on the adult population and sub-groups in Singapore. To address the pediatric issue, we have collaborated with a local pediatric hospital to perform a cross-sectional serological study using blood collected for other reasons. This would be the subject of subsequent research outside of the scope of this thesis.

The results of the following study are important to assist public health decision makers determine the extent of infection, the possible immunity in the population and therefore protection against subsequent 2009 H1N1 epidemic waves, and the consequent need for vaccination and for continued preparedness of public health and clinical services.

Study 3

Chen MIC, **Lee VJM**, Lim WY, Barr I, Lin RTP, Koh GCH, Yap J, Lin C, Cook AR, Laurie K, Tan LWL, Tan BH, Loh J, Shaw R, Durrant C, Chow VTK, Kelso A, Chia KS, Leo YS. 2009 Influenza H1N1 seroconversion rates and risk factors among distinct adult cohorts in Singapore. JAMA. 2010; 303:1383-91.

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2009 Influenza A(H1N1) Seroconversion Rates and Risk Factors Among Distinct Adult Cohorts in Singapore

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Context Singapore experienced a single epidemic wave of 2009 influenza A(H1N1) with epidemic activity starting in late June 2009 and peaking in early August before subsiding within a month.

Objective To compare the risk and factors associated with H1N1 seroconversion in different adult cohorts.

Design, Setting, and Participants A study with serial serological samples from 4 distinct cohorts: general population (n=838), military personnel (n=1213), staff from an acute care hospital (n=558), and staff as well as residents from long-term care facilities (n=300) from June 22, 2009, to October 15, 2009. Hemagglutination inhibition results of serum samples taken before, during, and after the epidemic and data from symptom questionnaires are presented.

Main Outcome Measures A 4-fold or greater increase in titer between any of the 3 serological samples was defined as evidence of H1N1 seroconversion.

Results Baseline titers of 40 or more were observed in 22 members (2.6%; 95% confidence interval [CI], 1.7%-3.9%) of the community, 114 military personnel (9.4%; 95% CI, 7.9%-11.2%), 37 hospital staff (6.6%; 95% CI, 4.8%-9.0%), and 20 participants from long-term care facilities (6.7%; 95% CI, 4.4%-10.1%). In participants with 1 or more follow-up serum samples, 312 military personnel (29.4%; 95% CI, 26.8%-32.2%) seroconverted compared with 98 community members (13.5%; 95% CI, 11.2%-16.2%), 35 hospital staff (6.5%; 95% CI, 4.7%-8.9%), and only 3 long-term care participants (1.2%; 95% CI, 0.4%-3.5%). Increased frequency of seroconversion was observed for community participants from households in which 1 other member seroconverted (adjusted odds ratio [OR], 3.32; 95% CI, 1.50-7.33), whereas older age was associated with reduced odds of seroconversion (adjusted OR, 0.77 per 10 years; 95% CI, 0.64-0.93). Higher baseline titers were associated with decreased frequency of seroconversion in community (adjusted OR for every doubling of baseline titer, 0.48; 95% CI, 0.27-0.85), military (adjusted OR, 0.71; 95% CI, 0.61-0.81), and hospital staff cohorts (adjusted OR, 0.50; 95% CI, 0.26-0.93).

Conclusion Following the June-September 2009 wave of 2009 influenza A(H1N1), 13% of the community participants seroconverted, and most of the adult population likely remained susceptible.

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On April 24, 2009, the World Health Organization (WHO) reported the emergence of a novel influenza A virus (2009 influenza A[H1N1]).[1] Early data from Mexico based on laboratory-confirmed cases suggested higher infection rates in younger age groups but higher case-fatality ratios in elderly individuals,[2] although it was initially unclear whether these observations were affected by biases in case ascertainment. Various experts have called for serological investigations to more accurately determine infection rates, especially since a substantial proportion of influenza infections are asymptomatic.[3]

Singapore, a Southeast Asian tropical city-state of 4.8 million people and a global travel hub, detected its first imported cases of 2009 influenza A(H1N1) in late May 2009. Virological surveillance documented sustained community transmission from the latter half of June 2009,[4-7] followed by a single epi-

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demic wave peaking in the first week of August and subsiding by September 2009.[7,8] We initiated a cohort study using serial blood specimens to determine antibody levels against 2009 influenza A(H1N1) as a marker of infection in 3 different population groups of public health concern—military personnel, acute care hospital workers, and staff members and residents of long-term care facilities and compared them with community-dwelling adults. The study aimed to compare the risk of infection in these different cohorts and to investigate risk factors for infection.

METHODS

Study Design

This was a cohort study including 4 different populations in Singapore and involving the planned collection of up to 3 serial serological samples from each individual: a baseline sample was collected either before the local 2009 influenza A(H1N1) epidemic using banked samples or in the early epidemic phase before widespread community transmission; the second sample was collected during the epidemic about 4 weeks after the epidemic had peaked; and third sample was collected at least 4 weeks after epidemic activity had subsided.

When possible, the start and stop dates of specimen collection across the cohorts were intentionally synchronized to allow intercohort comparison of seroconversion rates at each follow-up time point. Clinical symptom reviews were performed using a standardized questionnaire once every 2 weeks for the community cohort and at each sample collection in the other 3 cohorts. Participants were asked to report all new-onset respiratory symptoms and constitutional symptoms such as headaches, myalgia, and fever (including measured temperature where available); and baseline demographic data and whether they had ever received seasonal influenza vaccination in the past.

Study Populations

1. Community-dwelling adults were recruited from the Multiethnic Cohort (MEC) of the Singapore Consortium of Cohort Studies (SCCS), a long-term research project initiated to study gene-environment interactions in chronic disease causation. The MEC (http://www.nus-cme.org.sg/home.html) is a subcohort of the SCCS, comprising about 9000 community-dwelling healthy Singaporeans aged 21 to 75 years, recruited through public outreach activities and referrals for which recruitment is ongoing. We enrolled new MEC recruits into the study (from late June 2009), and recontacted 2400 existing MEC participants, with the aim of enrolling 900 participants. For the first serum sample collection, new recruits donated fresh baseline blood, while existing participants granted permission to use specimens banked on original recruitment. Symptom questionnaires were administered via telephone interviews at 2-week intervals.

2. The military personnel cohort was recruited from the Singapore Armed Forces, Singapore's national military and composed largely of conscripted males who serve after completion of high school from ages 18 through 19 years. Most individuals reside in military camps during weekdays but return to the community on weekends. Individuals were recruited by invitation from 15 units selected to give a good representation of the entire military structure, with a total personnel of 1570. Blood samples were taken at all 3 time points together with self-administered questionnaires.

3. Hospital staff from Tan Tock Seng Hospital, an acute care hospital with 6000 staff members, formed the third cohort. Staff members were recruited through e-mail notifications and by word-of-mouth referrals. Blood samples were taken at all 3 time points along with self-administered questionnaires. Information on symptomatic episodes was augmented through sickness absenteeism records for details such as dates of illness.

4. Staff and residents from 2 long-term care facilities, Jamiyah Home for the Aged and Peacehaven Nursing Home, were recruited by invitation. Between the 2 facilities are a total of 179 staff members and 520 residents (200 residents were able to give consent) who rarely go outside the facility. In this cohort, only the first and third serum samples were taken, with questionnaires simultaneously administered by trained interviewers.

Specimen Collection and Laboratory Methods

Venous blood was taken in 5- to 10-mL plain tubes. Serum samples were pretreated with receptor destroying enzyme (RDE [II], Deka Seiken Co Ltd, Tokyo, Japan), 1:4 (vol/vol), at 37°C for 16 hours, before enzyme inactivation by the addition of an equal volume of 1.6% trisodium citrate (Ajax Chemicals, Melbourne, Australia) and incubation at 56°C for 30 minutes.

The hemagglutination inhibition assay was performed according to standard protocols at the World Health Organization Collaborating Centre for Reference and Research on Influenza in Melbourne, Australia.[9] Egg-grown A/California/7/2009 A(H1N1) pandemic virus was purified by sucrose gradient, concentrated and inactivated with β-propiolactone, to create an influenza zonal pool preparation (a gift from CSL Limited, Melbourne, Australia). Twenty-five microliters (4 hemagglutination units) of influenza zonal pool-A/California/7/2009 virus was incubated at room temperature with an equal volume of RDE-treated serum. Serum samples were titrated in 2-fold dilutions in phosphate-buffered saline from 1:10 to 1:1280. Following 1 hour of incubation, 25 µL of 1% (vol/vol) turkey red blood cells was added to each well. Hemagglutination inhibition was read after 30 minutes. Titers were expressed as the reciprocal of the highest dilution of serum where hemagglutination was prevented. We defined seroconversion as a 4-fold or greater increase in antibody titers.

The hemagglutination inhibition assay was assessed on paired serum samples from 56 cases of 2009 influenza A(H1N1) that were confirmed through reverse transcription–polymerase chain reaction (RT-PCR): 28 participants from this

cohort study, plus 7 outbreak-related cases and 21 clinical cases admitted to Tan Tock Seng Hospital. Forty-five patients (80%) seroconverted to the pandemic strain, A/California/7/2009. Only 20 patients (20%) seroconverted to A/Brisbane/59/2007(H1N1) and 7 (13%), to A/Wisconsin/15/2009(H3N2).

Data Analysis and Statistics

Participants who seroconverted between any successive pairs of blood specimens (either from baseline to the second sample, second to third sample, or first to third sample) were considered as ever having had serological evidence of infection during the study period. Geometric mean titers (GMTs) were estimated by assigning a value of 5 for titers lower than 10 and a value of 1280 for titers of 1280 or higher.

Episodes of acute respiratory illness were defined as new-onset illness with any respiratory symptoms of rhinorrhea, nasal congestion, sore throat, or cough; and febrile respiratory illness was defined as an acute respiratory episode with self-reported fever or a body temperature of 37.5°C or higher. The date of each illness episode was the earliest symptom onset date or sickness absenteeism if onset dates were unavailable. Febrile respiratory illness episodes that preceded seroconversion were graphed by illness date against influenza epidemic activity. Likewise, illness episodes preceding seroconversion were used to estimate the proportion of seroconverting individuals with acute respiratory or febrile respiratory illness episodes. Singapore influenza epidemic activity data were from laboratory surveillance on the weekly proportion of influenza-like illness general practice samples testing positive for 2009 influenza A(H1N1) and the weekly number of influenza-like illness consults seen by a separate sentinel general practice network[7,8]—the 2 data sets were multiplied to give a weekly epidemic curve.

As some participants formed natural groupings, such as households and military units, and as contagious disease status is nonindependent within groups, we accounted for nonindependence using dummy variables corresponding to disease status of others within the group. For the community cohort, we introduced 2 indicator variables coding for 3 categories corresponding to known seroconversion status for other individuals in the household—at least 1 other household member seroconverted, no one else in the household seroconverted, or other permutations (no other household member in the study or other members in the study but seroconversion not known). For military camps, a variable indicating the proportion of the other unit members who seroconverted was introduced. The same was done for hospital staff, using functional operating units (75 wards or departments). We then performed

Table 1. Cohort Characteristics

	Community	Military	Hospital Staff	Long-term Care
Timing of blood draws in 2009				
Baseline	June 22-27[a]	June 22-July 1	June 22-July 7	July 17-27
Second	August 20-29	August 20-September 3[b]	August 19-September 3	NA
Third	October 6-11	September 29-October 9	September 29-October 15	October 5-7
Samples, No. (%) of participants				
Baseline	838 (100)	1213 (100)	558 (100)	300 (100)
Second	621 (74)	920 (76)	501 (90)	NA
Third	689 (82)	776 (64)	467 (84)	250 (83)
All 3 samples	583 (70)	636 (52)	431 (77)	NA
≥2	727 (87)	1060 (87)	537 (96)	250 (83)
No. (%) of reviews completed[c]	4766 (95)	1680 (69)	1098 (98)	250 (83)
Age, mean (range), y	43 (21-74)	22 (17-62)	34 (20-67)	56 (18-109)
Age in years, No. (%)				
15-19	0	554 (46)	0	12 (4)
20-24	92 (11)	473 (39)	110 (20)	28 (9)
25-29	66 (8)	93 (8)	129 (23)	24 (8)
30-39	152 (18)	44 (4)	164 (29)	47 (16)
40-49	298 (36)	31 (3)	96 (17)	14 (5)
50-59	166 (20)	14 (1)	52 (9)	32 (11)
≥60	64 (8)	4 (<1)	7 (1)	143 (48)
Sex, No. (%)				
Male	353 (42)	1175 (97)	92 (16)	131 (44)
Female	485 (58)	38 (3)	466 (84)	169 (56)
Seasonal influenza vaccine, No. (%)				
No	729 (87)	696 (57)	52 (9)	160 (53)
Yes	109 (13)	517 (43)	506 (91)	140 (47)

Abbreviation: NA, not applicable.
[a] Specimen collection dates for 23 community cohort participants; baseline samples for the remaining 815 participants used specimens banked on original recruitment into ongoing research study on chronic disease causation.
[b] Excludes 11 samples taken on September 9 and 10, 2009.
[c] Denominator is based on baseline multiplied by number of scheduled follow-up reviews: 6 for community, 2 for military, 2 for hospital staff, 1 for long-term care.

univariate and multivariate logistic regression using these dummy variables alongside baseline titer, age, sex, and seasonal influenza vaccine status to assess their contribution to seroconversion; odds ratios (ORs) with asymptotic Wald 95% confidence intervals (CIs) and 2-sided *P* values are presented with statistical significance set at the .05 level.[10] Multivariate analysis involved stepwise logistic regression, wherein variables that did not improve model fit at $P < .10$ were discarded.

A sample size of 450 participants per cohort was needed to give a power of 90% to detect (with a 2-sided *P* value of <.05) seroconversion rates that were 10% higher for a given cohort than the community sample, which was assumed would have seroconversion rates of 20% to 30% (similar to the 1957 pandemic[11]). Target sample sizes were 600 for hospital staff and long-term care facility cohorts and 900 for the community cohort to allow for loss to follow-up rates of 25% and 50%, respectively. The military cohort was substantially larger to allow comparison of seroconversion rates in different military units.

Where appropriate, 95% CIs for proportions were computed using the Wilson score-based method.[12,13] All statistical analyses were performed using STATA 10.0 (StataCorp, College Park, Texas).

Ethics Review

Written informed consent was obtained from all participants. The study was approved by the ethics review boards of the National Healthcare Group, Singapore Armed Forces, and National University of Singapore.

RESULTS

TABLE 1 describes the 4 cohorts. We completed baseline collection from 838 community participants by June 27, 2009, 1213 military participants by July 1, 2009, and 558 hospital participants by July 7, 2009, after simultaneously starting recruitment on June 22, 2009. The community cohort–banked samples dated back to June 2005, with 790 of 838 specimens (94%) collected before May 26, 2009, when the first imported influenza 2009 A(H1N1) case was detected in Singapore.[4] Logistical difficulties delayed baseline collection of the 300 long-term care facilities cohort participants until July 27, 2009, but there were no confirmed cases or excess influenza-like illness in either long-term care facility before the collection date. All participants (except those from the long-term care facilities cohort) were recalled for the second sample collection between August 19 and September 3, 2009, and the third sample collection between September 29 and October 15, 2009. In each cohort, 80%

Table 2. Baseline Titers by Cohorts, by Seasonal Influenza Vaccination in All Cohorts, and by Age Groups in the Community Cohort

	No. of Participants	Distribution of Antibody Titers, No. (%)			GMT (95% CI)	*P* Value
		<10[a]	10-20	≥40		
Cohort						
Community	838	738 (88)	78 (9)	22 (3)	5.8 (5.6-6.0)	
Military	1213	921 (76)	178 (15)	114 (9)	7.4 (7.1-7.7)	<.001[b]
Hospital staff	558	351 (63)	170 (30)	37 (7)	7.6 (7.2-8.1)	<.001[b]
Long-term care facilities	300	252 (84)	28 (9)	20 (7)	6.4 (6.0-6.9)	.007[b]
Seasonal influenza vaccine						
Community						
No	756	666 (88)	73 (10)	17 (2)	5.8 (5.6-6.0)	.34[c]
Yes	82	72 (88)	5 (6)	5 (6)	6.1 (5.3-7.1)	
Military personnel						
No	696	538 (77)	91 (13)	67 (10)	7.4 (6.9-7.9)	.98[c]
Yes	517	383 (74)	87 (17)	47 (9)	7.4 (6.9-7.9)	
Hospital staff						
No	52	39 (75)	11 (21)	2 (4)	6.4 (5.6-7.4)	.07[c]
Yes	506	312 (62)	159 (31)	35 (7)	7.8 (7.3-8.3)	
Long-term care facilities						
No	160	141 (88)	9 (6)	10 (6)	6.1 (5.5-6.7)	.14[c]
Yes	140	111 (79)	19 (14)	10 (7)	6.8 (6.1-7.7)	
Age groups in community cohort, y						
20-24	92	73 (79)	14 (15)	5 (5)	6.7 (5.8-7.7)	.002[d]
25-29	66	53 (80)	10 (15)	3 (5)	6.6 (5.6-7.7)	
30-39	152	136 (89)	14 (9)	2 (1)	5.6 (5.3-6.0)	
40-49	298	270 (91)	20 (7)	8 (3)	5.7 (5.4-6.0)	
50-59	166	148 (89)	15 (9)	3 (2)	5.7 (5.3-6.1)	
≥60	64	58 (91)	5 (8)	1 (2)	5.5 (5.0-6.1)	

Abbreviations: CI, confidence interval; GMT, geometric mean antibody titers.
[a] No detectable antibodies.
[b] Compared with community cohort using unpaired *t* test.
[c] Participants who did not have seasonal influenza vaccine compared with those who did using unpaired *t* test.
[d] Using linear regression with age as an explanatory value for GMT.

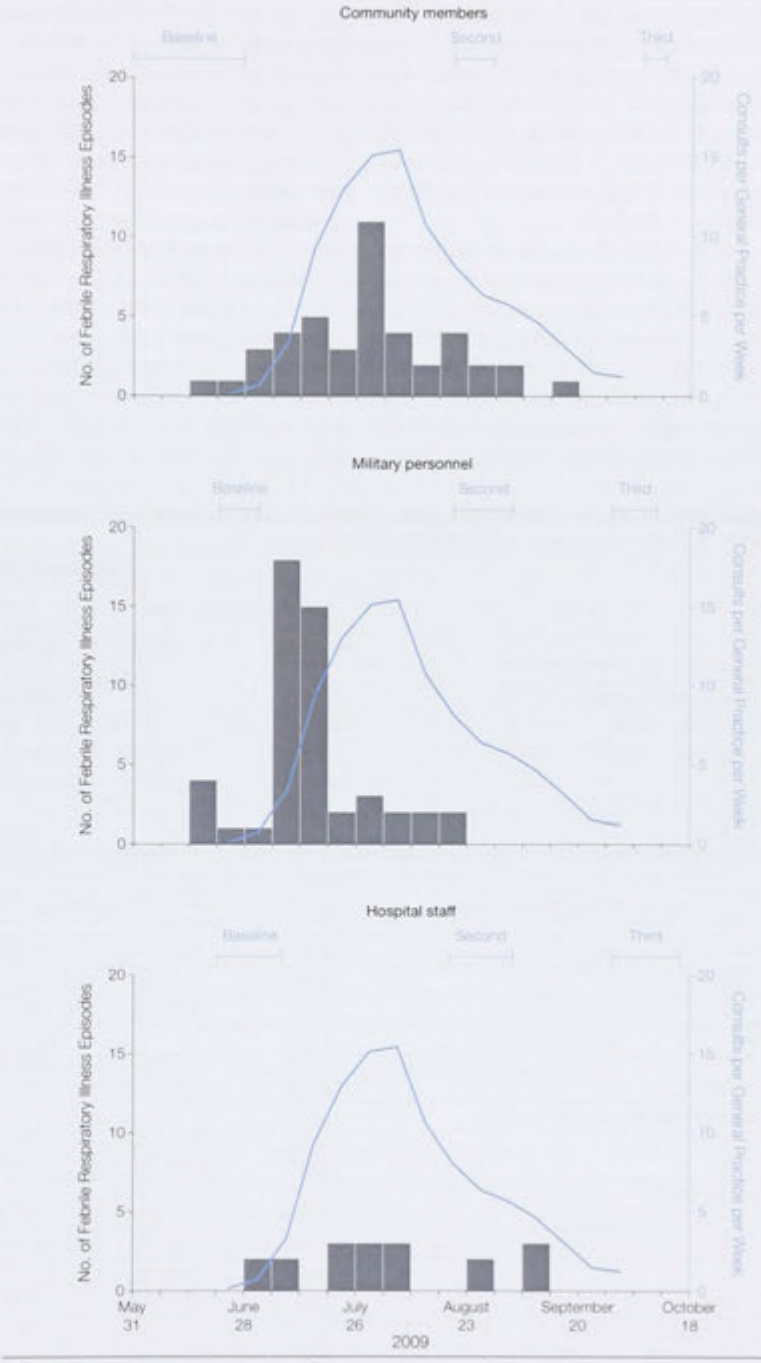
or more returned for at least 1 follow-up sample so that, except for the long-term care facilities cohort, the final number of participants for which seroconversion data was available exceeded our targeted sample sizes. Scheduled follow-up symptom reviews were also reasonably complete except in the military for which follow-up reviews were restricted to those with follow-up blood samples.

Military personnel were a mean age of 22 years (range, 17-62 years); hospital staff, 34 years (range, 20-67 years); and the community cohort, 43 years (range, 21-74 years), whereas the long-term care cohort, of which 54% (162/300) were residents, were a mean age of 56 years (range, 18-109 years). Sex distributions reflect the predominantly male workforce in the military (97%, 1175/1213) and female workforce in the hospital staff (84%, 466/558). Only 13% (109/838) in the community cohort had previously ever received seasonal influenza vaccine compared with 91% (506/558) of hospital staff, 43% (517/1213) of military, and 47% (140/300) of participants from long-term care facilities.

The baseline GMT for hospital staff was 7.6 (95% CI, 7.2-8.1); military personnel, 7.4 (95% CI, 7.1-7.7); and staff and residents of long-term care facilities, 6.4 (95% CI, 6.0-6.9), all of which were significantly higher than those of the community cohort: 5.8 (95% CI, 5.6-6.0; TABLE 2). The GMT of in-hospital staff who had received a seasonal influenza vaccine was 7.8 (95% CI, 7.3-8.3); whereas the GMT of staff who had not received the seasonal vaccine was 6.4 (95% CI, 5.6-7.4; $P = .07$). In the largely unvaccinated community cohort, younger age was significantly associated with higher baseline titers ($P = .002$).

The FIGURE shows that the epidemic curve peak for the community cohort coincided with the national peak in influenza epidemic activity, whereas the military personnel epidemic peaked 2 to 3 weeks earlier. Seroconversion occurred mostly between the baseline and the second sample for the community

Figure. Epidemic Curves for Each Cohort Constructed From Febrile Respiratory Illness Episodes in Seroconverting Participants



Braces represent the sampling periods and are compared against influenza epidemic activity as observed through H1N1-2009 general practice sentinel data. The H1N1-2009 general practice sentinel surveillance data are constructed by multiplying the proportion of laboratory surveillance isolates that tested positive for H1N1-2009 from the Ministry of Health and the number of influenza-like illness consults per general practice from a sentinel general practice network,[7,8] which gives the estimated number of general practice influenza-like illness consults that are influenza 2009 A(H1N1) for that week. Epidemic activity appears to have peaked in the week starting on August 2, 2009, at an estimated 15.5 consults per general practice per week.

with 70 of 98 eventual seroconversions (71%; 95% CI, 62%-79%) and for the military cohorts with 254 of 312 seroconversions (81%; 95% CI, 77%-85%) compared with hospital staff with 16 of 35 seroconversions (46%; 95% CI, 30%-62%) (TABLE 3). In the long-term care facilities cohort, only 3 of 250 (1.2%; 95% CI, 0.4%-3.5%) seroconverted, so this cohort was omitted from additional analysis.

Table 3 also shows the proportions of those who seroconverted as an indicator of the variation in risk of infection. In the community cohort, 13% seroconverted vs 29% in the military and 7% in the hospital staff cohort. Community participants aged 20 through 24 years were at higher risk than older participants with 21% of those in community and 24% of those in the military cohorts seroconverting vs 8% of those 60 years or older in the community cohort. Furthermore, 44% of those aged 15 through 19 years in the military cohort seroconverted. No discernible effect from prior seasonal influenza vaccination existed except for the military cohort, for which 37% of unvaccinated participants seroconverted vs 19% of those vaccinated. Participants with higher baseline titers had lower seroconversion rates—13% of military participants with titers of 40 or higher seroconverted vs 32% with titers lower than 10. Seroconversion data were available for 223 participants residing in the 106 households in the community cohort. Twenty-nine percent (10/34; 95% CI, 17%-46%) of those living with another household member who was known to have seroconverted vs 12% (23/189; 95% CI, 8%-18%) of those living in households in which no one else had seroconverted and 13% (65/504; 95% CI, 10%-16%) in other community participants for whom seroconversion data for other household members were not available had seroconversion. Because there was no significant difference in seroconversion rates for the latter 2 groups ($P = .79$), these were combined during multivariate analysis.

On multivariate analysis (TABLE 4), having another household member who seroconverted remained associated with

Table 3. Factors Associated With Seroconversion by Cohorts

	Participants With Seroconversion by Cohort					
	Community Members		Military Personnel		Hospital Staff	
	No./Total	% (95% CI)	No./Total	% (95% CI)	No./Total	% (95% CI)
Detection of seroconversion by blood draw						
Baseline to second blood draw	70/621	11 (9-14)	254/920	28 (25-31)	16/501	3 (2-5)
Second to third	16/584	3 (2-4)	21/636	3 (2-5)	12/432	3 (2-5)
Baseline to third	83/690	12 (10-15)	223/776	29 (26-32)	32/468	7 (5-9)
Ever	98/727	13 (11-16)	312/1060	29 (27-32)	35/537	7 (5-9)
Age, y[a]						
15-19			115/259	44 (38-50)		
20-24	16/78	21 (13-31)	96/399	24 (20-28)	6/104	6 (3-12)
25-29	5/50	10 (4-21)	11/75	15 (8-24)	9/123	7 (4-13)
30-39	18/132	14 (9-21)	1/37	3 (0-14)	7/157	4 (2-9)
40-49	43/267	16 (12-21)	0/28	0 (0-12)	10/95	11 (6-18)
50-59	12/147	8 (5-14)	1/11	9 (2-38)	2/51	4 (1-13)
≥60	4/53	8 (3-18)	1/4	25 (5-70)	1/7	14 (3-51)
Sex[a]						
Male	45/295	15 (12-20)	308/1028	30 (27-33)	5/90	6 (2-12)
Female	53/432	12 (10-16)	4/32	13 (5-28)	30/447	7 (5-9)
Seasonal influenza vaccine[a]						
No	87/659	13 (11-16)	227/616	37 (33-41)	1/50	2 (0-10)
Yes	11/68	16 (9-27)	85/444	19 (16-23)	34/487	7 (5-10)
Baseline titers[a]						
<10	93/631	15 (12-18)	252/799	32 (28-35)	27/340	8 (6-11)
10	2/48	4 (1-14)	30/91	33 (24-43)	8/126	6 (3-12)
20	3/27	11 (4-28)	17/71	24 (16-35)	0/36	0 (0-10)
≥40	0/21	0 (0-15)	13/99	13 (8-21)	0/35	0 (0-10)
Other household member[a,b]						
≥1	10/34	29 (17-46)				
No one else	23/189	12 (8-18)				
Other	65/504	13 (10-16)				

Abbreviation: CI, confidence interval.

[a] Numerator is individuals who had ever seroconverted; denominator is individuals who had at least 1 follow-up sample (second, third, or both samples).

[b] Other household member seroconverted: at least 1 other household member with seroconversion, no one else in household with seroconversion, and other combinations (no other household member in the study or other members in the study but seroconversion data not available).

a higher likelihood of infection (adjusted OR, 3.32; 95% CI, 1.50-7.33). The proportion within the unit who had seroconverted was associated with increased risk of infection in the military (adjusted OR, 1.42; 95% CI, 1.27-1.59) but not among hospital staff. After adjusting for infections in the same military unit, vaccination and sex were no longer significant, but older age remained significantly protective (adjusted OR, 0.42 per 10 years; 95% CI, 0.27-0.65), similar to the community cohort (adjusted OR, 0.77 per 10 years; 95% CI, 0.64-0.93). Higher baseline titers had lower likelihood of seroconversion in the community (adjusted OR, 0.48; 95% CI, 0.27-0.85), hospital staff (adjusted OR, 0.50; 95% CI, 0.26-0.93), and military cohorts (adjusted OR, 0.71; 95% CI, 0.61-0.81).

During the study period, acute respiratory and febrile respiratory illness episodes were more common for individuals who seroconverted. In community participants, 73% (72/98; 95% CI, 64%-81%) of those who had seroconverted reported 1 or more acute respiratory illness episodes vs 43% (269/629; 95% CI, 39%-47%) of those who had not ($P<.001$), and 44% (43/98; 95% CI, 34%-54%) of those who had seroconverted had febrile respiratory illness episodes vs 9% (56/629; 95% CI, 7%-11%) of those who had not ($P<.001$). Among hospital staff, 69% (24/35; 95% CI, 52%-81%) of those who had seroconverted had acute respiratory illness vs 15% (75/502; 95% CI, 12%-18%) of those who had not ($P<.001$), and 51% (18/35; 95% CI, 36%-67%) of those who had seroconverted had febrile respiratory illness vs 8% (41/502; 95% CI, 6%-11%) of those who had not ($P<.001$). The military cohort reported lower acute respiratory illness and febrile respiratory illness rates: 31% (98/312; 95% CI, 27%-37%) of those who had seroconverted had acute respiratory illness vs 24% (181/748; 95% CI, 21%-27%) of those who had not ($P=.02$), and 16% (50/312; 95% CI, 12%-21%) of those who had seroconverted had febrile respiratory illness vs 7% (56/748; 95% CI, 6%-10%) of those who had not ($P<.001$).

COMMENT

To our knowledge, this is the first cohort study designed to estimate the extent of infection with 2009 influenza A(H1N1) using serological assays. Our study shows that at the end of the first epidemic wave in Singapore a substantial proportion of the Singapore adult population lack antibodies to the novel strain, with only 13% of the community cohort having serological evidence of infection. This infection rate estimate is compatible with the 11% clinical attack rate for Singapore estimated from influenza-like illness reporting[7] and was fairly similar to estimates of adult incidence from a cross-sectional serological study conducted after the first epidemic wave of 2009 influenza A(H1N1) in the United Kingdom.[14]

Our study also shows the variation in infection risks, with younger age groups and military personnel having much higher infection rates. The lower infection rates in older participants corroborate other epidemiological observations.[14,15] Because there was no 15- to 19-year age group in the community cohort, we are unable to conclude whether higher infection rates in the military were due to the younger age or increased transmission, although historical pandemic data[16] and the strong association between infection risk and level of intraunit infections in our study

Table 4. Univariate and Multivariate Analysis for Factors Associated With Seroconversion in Community, Military, and Hospital Staff

	Crude OR (95% CI)	*P* Value	Adjusted OR (95% CI)	*P* Value
Community				
Age per 10 y	0.81 (0.67-0.97)	.02	0.77 (0.64-0.93)	.007
Female sex	0.78 (0.51-1.19)	.25		
Had seasonal influenza vaccine	1.27 (0.64-2.51)	.50		
Baseline titer[a]	0.54 (0.31-0.94)	.03	0.48 (0.27-0.85)	.01
Other household member[b]	2.86 (1.33-6.19)	.007	3.32 (1.50-7.33)	.003
Military				
Age per 10 y	0.25 (0.15-0.41)	<.001	0.42 (0.27-0.65)	<.001
Female sex	0.33 (0.12-0.96)	.04		
Had seasonal influenza vaccine	0.41 (0.30-0.54)	<.001		
Baseline titer[a]	0.76 (0.66-0.87)	<.001	0.71 (0.61-0.81)	<.001
Proportion in unit (per 10%)[c]	1.56 (1.40-1.72)	<.001	1.42 (1.27-1.59)	<.001
Hospital				
Age per 10 y	1.06 (0.76-1.46)	.74		
Female sex	1.22 (0.46-3.24)	.69		
Had seasonal influenza vaccine	3.68 (0.49-27.46)	.20		
Baseline titer[a]	0.50 (0.26-0.93)	.03	0.50 (0.26-0.93)	.03
Proportion in unit per 10%[c]	1.24 (0.83-1.85)	.30		

Abbreviations: CI, confidence interval; OR, odds ratio.
[a] For every unit increase in baseline titer, for which the integer values 0 to 8 denote titers of <10, 10, 20, 40, 80, 160, 320, 640, and 1280 or more, respectively.
[b] Had at least 1 other household member who seroconverted compared with all other community participants (including those from households for which no one else seroconverted, no other household member in the study, or other members in the study but seroconversion data not available).
[c] Proportion in unit who seroconverted.

point to greater transmission intensity in military populations. This suggests that special preventive measures in military subpopulations may be justified in the event of influenza epidemics. The increased risk of infection for community participants from households in which at least 1 other member seroconverted was expected, although it was not possible to determine the direction of transmission in this study.

In contrast, hospital staff and the long-term care facilities cohorts had lower infection rates. Besides the high baseline titers, hospital staff may also have been protected at work because of intense patient and visitor screening, use of personal protective equipment, and other infection control measures deployed during the epidemic.[17,18] Such combination strategies may help prevent influenza transmission, although it is difficult to attribute the specific effect of these interventions without control groups. Staff and residents of the long-term care facilities may likewise have been protected by similar measures, but other factors such as reduced host susceptibility in the older age groups should be considered, for as others have found, long-term care facilities were largely spared from 2009 influenza A(H1N1) outbreaks.[19] Because large segments of these populations lacked antibodies after the initial epidemic wave, outbreaks might occur in subsequent epidemic waves. Likewise, only 13% of the community cohort seroconverted, which supports the case for targeted vaccination in populations for which protection is desired.

In both the community cohort and hospital staff, about half the participants who seroconverted reported a febrile respiratory illness episode. This is comparable with estimates of influenza-like illness proportions among serologically confirmed influenza cases from seasonal influenza studies.[20,21] Febrile respiratory illness episodes were less common among nonseroconverters, showing that febrile respiratory illness is reasonably specific (but not very sensitive) for influenza during epidemics.[22] The large number of community participants with acute respiratory illness episodes who did not seroconvert may have had other infections; rhinovirus circulates throughout the year and is the most common identifiable cause of acute respiratory illness in Singapore.[23,24] There were proportionately fewer febrile respiratory illness episodes in military personnel possibly due to underreporting for which illness data were based solely on self-administered questionnaires.

Using serological cohorts is one of the best ways to estimate infection rates, particularly for large outbreaks such as 2009 influenza A(H1N1) for which laboratory confirmation cannot be performed for most cases. Our cohort study demonstrates that those with higher baseline titers have significantly lower infection rates, perhaps indicative of protection against 2009 influenza A(H1N1) infection. Our study also suggests that baseline circulating antibodies to 2009 influenza A(H1N1) exist in individuals without clinical evidence of prior infection (Table 2). Baseline antibody titers were marginally higher in vaccinated hospital staff, compatible with findings that 12% to 22% of adults experienced a 4-fold or greater increase in antibody titers to 2009 influenza A(H1N1) after seasonal influenza vaccination.[25] Our findings on age-specific prevalence of baseline antibodies are similar to those from China where only 1.7% of adults (serum samples collected July-August 2008) had preexisting antibody titers of at least 40 to 2009 influenza A(H1N1) on hemagglutination inhibition assay, with even lower responses in those 60 years or older.[26] In contrast, Hancock et al[25] found that baseline antibodies were more prevalent in older adults in the United States, suggesting that further studies are needed to understand whether the discrepant observations are due to seasonal H1N1 vaccination,[26] exposure to influenza, or other community-specific factors. Notably, in community participants aged 65 years or older (for whom vaccination is recommended), only 11% reported ever having received influenza vaccination. This corroborates previous estimates that influenza vaccine uptake in Singapore remains low.[27]

One limitation of our study is the lack of a pediatric population due to the difficulty in obtaining serial blood specimens in this age group; cross-sectional surveys using residual samples may be more feasible for estimating childhood infection rates. Furthermore, our community cohort may not be truly representative of the Singapore population because it largely comprised healthy volunteers. Although these factors preclude us from determining the actual infection rate in Singapore, our study allows us to refine estimates on the numbers at risk, obtain better case fatality rate estimates in adult age groups, and inform policy on vaccination. Finally, apart from the community cohort, the baseline collection started after influenza 2009 A(H1N1) had begun to circulate, albeit at low levels. However, subanalysis of the military and hospital cohorts found no evidence of higher baseline titers in participants whose baseline samples were collected later.

In conclusion, our study shows wide variation in serologically determined infection rates by cohorts and age groups, suggesting that context-specific risks of infection need to be taken into account and that interventions need to be tailored to the population at risk. Although it appears that a large proportion of the Singapore adult population remain susceptible to the 2009 influenza A(H1N1) virus after the first epidemic wave, for a significant second wave to occur, a sufficient number of susceptible children may also be required for efficient transmission. These and other factors will need to be considered in the determination of optimal pandemic vaccination strategies for influenza A(H1N1).[28]

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Chapter Six

Clinical Features of Influenza in Closed and Semi-closed Environments

The previous seroepidemiology study showed that the military cohort had a substantially higher seroconversion rate compared to the general population. From previous studies, closed and semi-closed environments such as schools, militaries, and boarding facilities have shown the propensity for high attack rates. These are possible conduits of spread of influenza due to living and/or working in close proximity. This is not only unique to the 2009 pandemic, but also in other influenza epidemics across time. For example, on board one military ship, despite having a high influenza vaccination rate among the sailors of more than 95%, an antigenically distinct H3N2 influenza virus resulted in a 42% attack rate within three weeks and substantial lost workdays which are critical on a ship at sea (1). In another military setting, influenza outbreaks affected up to 58% of the population in less than a month (2). Our previous study from March 2006 and March 2007 performed in the Singapore military found that influenza A accounted for about 24% and influenza B 12% of all acute respiratory infections (3). Other similar closed environments have also yielded high attack rates from influenza, as shown in one boarding school for girls in the United Kingdom which reported a 71% overall attack rate during an influenza outbreak (4).

Schools also have high transmission and attack rates among children which may be due to their increased susceptibility to infection and the close interaction during various activities within the school environment, similar to the military setting. During the initial months of the 2009 pandemic, there were also several instances of substantial transmission in schools – in New York, United States, 33% of students in one school reported having influenza-like symptoms (5). At a vocational boarding school in China, the overall infection rate was 32%, and risk factors for infection included sharing a classroom and dormitory space (6). In Osaka, Japan, reports of an

outbreak of the 2009 H1N1 pandemic virus in about 100 children in a school, together with transmission to other schools, prompted mass school closures across the city (7). Schools are also equally affected during seasonal influenza epidemics. One earlier mathematical modeling study in Taiwan suggested that the reproductive number (R_0) - which is the average number of secondary cases that originate from an infected primary case in the absence of immunity - ranged from 2.8 to 16.9 in school settings, and was substantially higher than the 1.2 to 2.4 in various community settings (8). Another seasonal influenza epidemic in 2005/2006 in England resulted in an overall attack rate in schools of 24.1%, ranging from 14.6% to 44.9% across different regions within the country (9). In Singapore during the 1968 pandemic, a study at the National University of Singapore found an attack rate of 19.2%, ranging from 12.8% in female students to 36.4% in adult non-academic staff (10).

Militaries have large populations across the world, often in closed or semi-closed settings which favor the rapid transmission of infectious diseases. The burden of respiratory infections such as influenza acquired in military populations is somewhat different from the community, due to the physical and mental challenges associated with military life (11). There is therefore a need to perform comprehensive surveillance for influenza in the military to understand the epidemiology and burden of disease, to guide preventive public health measures to reduce the impact.

In the Singapore context, the military is an ideal platform to study the impact of influenza due to the existence of excellent surveillance platforms, and an existing influenza pandemic preparedness and response plan which had been built upon the 2003 SARS outbreak in Singapore, together with guidance on influenza preparedness

and response from the WHO (12). This preparedness and response plan resulted in interventions to be studied in later chapters. The Singapore military's semi-closed environment and the conscript population which forms the majority of the population provides results that are likely to be applicable in other closed settings such as schools which are of concern to policy makers worldwide.

In addition, the military is important as a sentinel group for national surveillance. The previous study has shown that epidemics in the military can develop faster than the national epidemic, and military outbreaks can be detected earlier due to the living environment where most cases report to a single medical facility and outbreaks can easily be detected. In the 1968 pandemic in Singapore, early outbreaks were detected in similar closed environments in a military camp and an island fishing village (13,14).

This study therefore aims to explore the epidemiology of influenza including incidence and seasonality, and the overall effectiveness of measures to reduce the impact of influenza. It shows the surveillance of influenza in the military during and after the 2009 pandemic, when it was initially believed that the 2009 H1N1 pandemic strain would be the dominant strain. It also determines the clinical features of influenza compared to non-influenza cases, and may be useful for clinicians identifying cases for clinical management.

This study also reinforces the findings of Chapter Four in detailing the difficulties of using ILI to identify influenza cases for treatment or surveillance without laboratory diagnosis, although we did not evaluate the effectiveness of ILI as a surveillance tool.

The ILI definition has often been used for influenza surveillance and also by clinicians to determine possible influenza infection for clinical management. A recent study by Kasper and colleagues looked at the accuracy of ILI as a tool in predicting influenza infections and found that the sensitivity was 73.8%, specificity 43.0% PPV 39.5% and NPV 76.5% (15). Other studies have found that fever and cough were the best predictive symptoms of influenza (16,17). The study by Monto and colleagues also found, as we did, that sore throat was a negative predictor of influenza (16). It is therefore important to look at the possible predictors of influenza among a range of possible influenza symptoms in the local setting. At the same time, all of these studies are based on subjects that were selected due to fever and respiratory symptoms in a clinical setting, limiting the ability of these studies to be generalized to milder influenza cases. Future studies should be expanded to include mildly symptomatic influenza cases including those without fever or respiratory symptoms, with study designs that include general community participant and all patients presenting to clinical facilities.

Study 4

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A Clinical Diagnostic Model for Predicting Influenza among Young Adult Military Personnel with Febrile Respiratory Illness in Singapore

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Abstract

Introduction: Influenza infections present with wide-ranging clinical features. We aim to compare the differences in presentation between influenza and non-influenza cases among those with febrile respiratory illness (FRI) to determine predictors of influenza infection.

Methods: Personnel with FRI (defined as fever≥37.5°C, with cough or sore throat) were recruited from the sentinel surveillance system in the Singapore military. Nasal washes were collected, and tested using the Resplex II and additional PCR assays for etiological determination. Interviewer-administered questionnaires collected information on patient demographics and clinical features. Univariate comparison of the various parameters was conducted, with statistically significant parameters entered into a multivariate logistic regression model. The final multivariate model for influenza versus non-influenza cases was used to build a predictive probability clinical diagnostic model.

Results: 821 out of 2858 subjects recruited from 11 May 2009 to 25 Jun 2010 had influenza, of which 434 (52.9%) had 2009 influenza A (H1N1), 58 (7.1%) seasonal influenza A (H3N2) and 269 (32.8%) influenza B. Influenza-positive cases were significantly more likely to present with running nose, chills and rigors, ocular symptoms and higher temperature, and less likely with sore throat, photophobia, injected pharynx, and nausea/vomiting. Our clinical diagnostic model had a sensitivity of 65% (95% CI: 58%, 72%), specificity of 69% (95% CI: 62%, 75%), and overall accuracy of 68% (95% CI: 64%, 71%), performing significantly better than conventional influenza-like illness (ILI) criteria.

Conclusions: Use of a clinical diagnostic model may help predict influenza better than the conventional ILI definition among young adults with FRI.

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Introduction

Influenza infections result in a wide range of clinical presentations, from the classical influenza-like illness (ILI), to milder respiratory infections, and subclinical infections. Determining the clinical predictors of influenza infection is important for the diagnosis and management of patients presenting with respiratory illness, helping to guide appropriate antiviral therapy, and to avoid unnecessary antibiotic use. This is particularly important in the young adult population, which constitutes an economically productive age group whereby early treatment may reduce work absenteeism [1]. The recent 2009 H1N1 pandemic has shown that young adults have a higher infection rate compared to other age groups [2]. For essential public services such as the military, police, civil defence, and healthcare with substantial proportions of young adults, early recognition and treatment may reduce service disruptions.

There has been research describing the differences in symptoms between influenza and non-influenza cases. However, few have been performed in tropical countries, where a large proportion of

the world's population reside. Influenza morbidity and mortality in tropical countries like Singapore has been shown to be comparable to temperate countries [3,4]. Furthermore, there has also been substantial co-circulation of other etiologic agents that can similarly cause acute respiratory illnesses [5]. While two recent tropical studies sought to differentiate the symptoms of these clinical entities, they had only limited number of cases [6,7], and were based only on hospital attendances in the peri-pandemic period, where inclusion criteria might be atypical.

Using data from a respiratory disease sentinel surveillance system in the Singapore military, we compare the differences in clinical presentation between influenza and non-influenza cases in young adults with febrile respiratory illness to determine predictors of influenza infection and aid case management especially where laboratory confirmation is not possible.

Methods

Singapore is a city state in tropical South-East Asia with 5 million people, with all Singaporean males serving two years of military service after high school. These servicemen live in barracks-style accommodation during weekdays and return home during weekends, maintaining continued interaction between the military and the Singapore population.

The Singapore military began a sentinel respiratory disease surveillance program in 4 major camps, including a recruit training camp, on 11 May 2009 (epidemiological-week 19), just before community spread of pandemic H1N1 in late-June 2009 [8,9]. All personnel who visited the primary healthcare clinics in these camps during the main consultation hours with febrile respiratory illness (FRI)—defined as the presence of fever $\geq$37.5°C with cough or sore throat—were recruited. The use of FRI contrasts with the usual measure of influenza-like illness (ILI, defined as fever $\geq$38.0°C with cough or sore throat); our choice reflected the desire to capture other febrile cases that also result in substantial absenteeism; while limiting cases to those with fever as an indicator of potential severity and absenteeism.

Repeat visits for the same illness episode as assessed by the consulting physician were excluded to avoid double counting. Nasal washes, collected separately from each side of the nose, were taken from consenting participants by trained medical staff, collected in viral transport media, and sent to the laboratory within 24 hours. Nasal washes were used as they have been shown to be equally or more sensitive than other methods such as nasal or throat swabs, and nasopharyngeal aspirates, in the detection of respiratory infections such as influenza [10–12].

In addition, interviewer-administered questionnaires were completed during the medical consultation, collecting information on patient demographics and clinical features. A follow-up phone questionnaire was conducted 2 weeks after the initial consultation to determine symptoms present during the entire course of illness.

Written informed consent was obtained. The study was approved by the military's Joint Medical Committee for Research, and by the institutional review boards of the National University of Singapore, and the Australian National University.

Laboratory Methods

To determine the etiology, we used the multiplex PCR strategy based on the Resplex assays described below, and performed additional singleplex PCR assays to determine the influenza subtype.

Total nucleic acids were extracted from each specimen using the DNA minikit (Qiagen, Inc, Valencia, CA, USA) according to the manufacturer's instructions. Five µl of extract were tested with Resplex I and II (version 2.0, Qiagen, Inc., Valencia, CA, USA) for the presence of respiratory micro-organisms on the LiquiChip 200 Workstation, again according to the manufacturer's instructions. The Resplex I and II (version 2.0) assays are multiplex PCR assays coupled with bead array detection technology and can simultaneously detect and subtype 18 different viruses and bacteria including influenza A and influenza B [13–15].

Specimens that were Resplex II positive for influenza A were further subtyped with real-time PCR for H1 or H3 (Singapore Ministry of Health), or for pandemic H1N1 [16]. Briefly, five µl of total genetic extracts were tested with the one-step SuperscriptIII/Platinum Taq kit (Invitrogen, Carlsbad, CA, USA) following the manufacturer's instructions on either the LightCycler machine from Roche or the Applied Biosystems real-time PCR machine (7500).

Statistical Analysis

We compared differences in overall clinical presentation between influenza and all non-influenza FRI cases. Univariate comparison of demographic parameters, symptoms and signs was conducted using logistic regression to determine statistically significant parameters of interest. Potential confounding was addressed by performing multivariate analyses where characteristics found to be statistically significant in univariate analyses were entered into a multivariate logistic regression model to identify independent clinical predictors, with non-significant terms in the multivariate analysis dropped one at a time starting with the highest p-value. To address another source of potential confounding among the remaining variables, we assessed for interactions between these variables but none proved significant. All statistical analyses were performed using Stata 9.0 (Stata Corp., College Station, TX, USA) and R (R Core Development Team). All tests were conducted at the 5% level of significance, with no explicit adjustment for multiple comparisons; instead, where appropriate, we present the expected number of false positive findings under the assumption that all null hypotheses are correct, a strongly conservative assumption. We report odds ratios (OR) and corresponding 95% confidence intervals (CI) where applicable.

The final multivariate model for influenza versus non-influenza cases was used to build a predictive probability equation as a clinical diagnostic model to determine the likelihood of influenza infection given the clinical characteristics. For this we developed the receiver operating characteristic (ROC) curve whence the area under the ROC (AUC) was calculated and two cut-off points determined: one maximizing the sum of sensitivity and specificity, the other maximising specificity while keeping sensitivity at 90%. Ten-fold cross-validation was used to guard against over-fitting, with AUC, sensitivity and specificity scores averaged over the ten folds.

Results

A total of 2858 eligible subjects were recruited from 11 May 2009 to 25 Jun 2010. Of these 2858 subjects, 2717 (95.1%) completed the telephone follow-up. The average age was 21 years old (SD 3.2), and 2853 (99.8%) were male. Of the 2858 subjects, there were 821 influenza cases, of which 434 (52.9% of all influenza cases) were 2009 pandemic influenza A (H1N1), 58 (7.1%) seasonal influenza A (H3N2), 269 (32.8%) influenza B, and 10 (1.2%) seasonal influenza A (H1N1), with 6 co-infections and 44 unsubtypable.

There were a total of 70 influenza vaccine failures, defined as seasonal or pandemic influenza infections that occurred despite previous vaccination with the relevant seasonal or pandemic

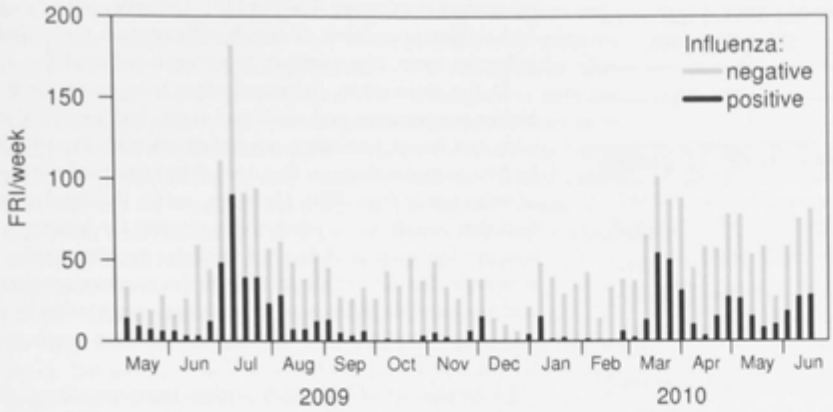

Figure 1. Weekly FRI cases, by influenza RT-PCR positivity, in 2009/10 in the Singapore military.
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vaccine respectively. Of these, there were 43 pandemic H1N1 vaccine failures, although 27 (63%) were vaccinated less than 2 weeks before onset of symptoms; 11 H3N2 vaccine failures, and 16 influenza B vaccine failures. There were no statistically discernible differences in influenza severity (fever ≥38.0°C or breathlessness) for vaccine failures compared to other influenza cases.

Figure 1 shows the number of FRI cases sampled per week, and the proportion of these cases that tested positive for influenza. For the non-influenza FRI cases, 289 (10.1% of all subjects) were diagnosed with coxsackie viruses/echoviruses, 247 (8.6%) rhinovirus, 217 (7.6%) H. influenzae, 130 (4.5%) coronaviruses, 76 (2.7%) parainfluenza viruses, 47 (1.6%) human metapneumovirus, 27 N. meningitidis, 12 S. pneumoniae, 5 adenoviruses, 2 RSV, and 1 bocavirus.

Clinical Features

Univariate analyses comparing the clinical features between influenza and non-influenza cases are presented in Figure 2, while the multivariate analyses adjusting for possible confounders are presented in Table 1.

From the univariate and multivariate analyses, influenza-positive cases were significantly more likely to present with running nose, chills and rigors, and higher temperature, and less likely to present with sore throat, photophobia, and injected pharynx, compared to influenza-negative cases (Figure 2 and Table 1). Ocular symptoms were significant on univariate but only marginally so on multivariate analysis, while nausea/vomiting was borderline significant on univariate but clearly significant on multivariate analysis. Based on the final model's maximum likelihood estimates, we created a diagnostic index that predicted influenza infection based on clinical presentation. The predicted probability of influenza infection (p_i) was calculated as follows:

$$10\ln\frac{p_i}{1-p_i} = -31 - 5[\text{sore throat}] + 6[\text{running nose}] + 2[\text{ocular symptoms}] - 3[\text{nausea/vomiting}] + 4[\text{chills/rigors}] - 7[\text{photophobia}] + 5[\text{fever}\geq 37.8] + 8[\text{fever}\geq 38] - 4[\text{injected pharynx}]$$

where [A] = 1 if the patient presents with that symptom or sign and 0 otherwise. A score (on the right hand side) of 0 corresponds to a 50% chance of influenza infection, -10 to about a 25% chance, -5 to about a 40% chance. The fever terms are cumulative, i.e. a fever of 37.9 adds 5 to the score, while a fever of 38.2 adds 13.

The AUC under ten-fold cross-validation was 69% (95% CI: 61%, 76%). Using a cut-off to maximize sensitivity and specificity, the model had sensitivity of 65% (95% CI: 58%, 72%), specificity of 69% (95% CI: 62%, 75%), and overall accuracy of 68% (95%

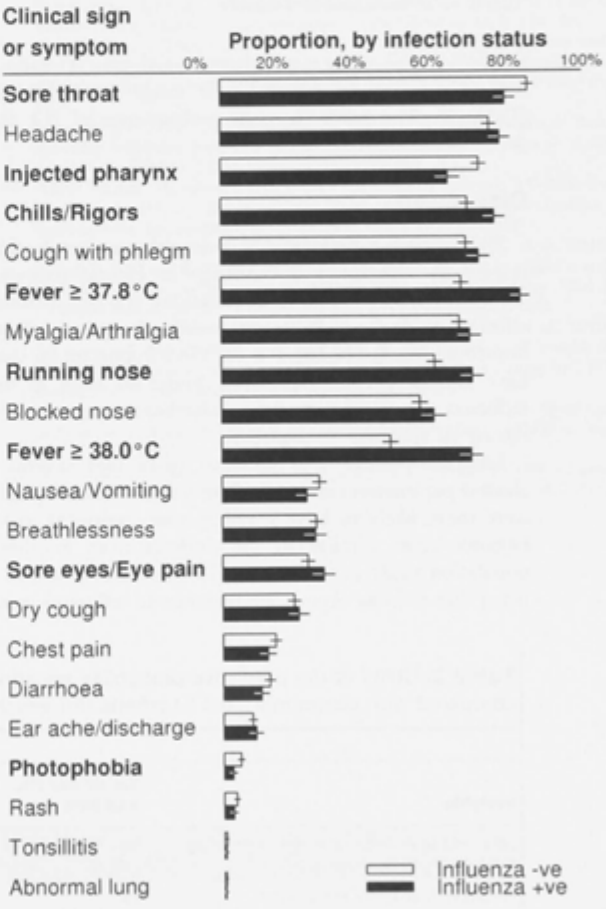

Figure 2. Univariate comparison of clinical signs or symptoms between influenza-positive and influenza-negative cases. Symptoms or signs are ranked by frequency for non-influenza cases. Empirical frequencies of presentation of each symptom or sign are presented in the right column as bars, with 95% confidence intervals represented by whiskers. Symptoms or signs that are statistically discernibly different at the 5% level are displayed in bold font. With 21 tests, the conservative expected number of false discoveries is 1.1.
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Table 1. Multivariate analysis comparing clinical features of influenza-positive with all influenza-negative FRI cases.

Influenza Positive vs Negative*		
Parameters	**Adjusted Odds Ratio (95% CI)**	**p value**
Sore throat	0.62 (0.48, 0.82)	<0.001
Running nose	1.86 (1.52, 2.29)	<0.001
Chills/rigors	1.52 (1.20, 1.91)	<0.001
Photophobia	0.49 (0.29, 0.83)	0.007
Fever (≥37.8°C)	1.64 (1.19, 2.26)	0.003
Fever (≥38°C)	2.15 (1.65, 2.80)	<0.001
Injected pharynx	0.69 (0.56, 0.86)	<0.001
Nausea/Vomiting	0.74 (0.59, 0.92)	0.007
Eye symptoms	1.25 (1.01, 1.55)	0.04

*Age, sore throat, running nose, sore eyes or eye pain, chills/rigors, photophobia, Fever ≥37.8°C, Fever ≥38.0°C, and injected pharynx were included in the analysis before non-significant terms were sequentially removed. With nine tests, the conservative expected number of false discoveries is 0.45.

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CI: 64%, 71%) under ten-fold cross validation. The model allows for differing cut-off specifications using the indicated criteria (Table 2). The relatively poor performance of ILI alone as a predictor is notable.

Discussion

Differentiating between influenza infections and other febrile respiratory illnesses is a challenge in clinical settings without laboratory assistance. In most situations, it is not feasible or cost-effective to perform PCR tests, while cheaper rapid tests have limited sensitivity [17,18]. It is therefore important for clinicians to have clinical presentation-based guides to assist in diagnosing influenza cases for treatment and further management, especially during an epidemic or pandemic.

Influenza-positive and negative cases had several differing clinical parameters. We have found that influenza-positive cases were more likely to have running nose compared to influenza-negative cases, similar to the findings from another general population study in the tropics [7]. This is contrary to previous belief that running nose is less common in influenza compared to other viral respiratory illnesses [19]. Likewise, influenza cases also had similar prevalence of cough with sputum compared to non-influenza cases, also contrary to previous belief [19].

At the same time, influenza cases were more likely to have higher temperature and chills and rigors but less likely to present with sore throat, providing supporting evidence to a previous study by Monto and colleagues that one of the most predictive symptoms of influenza is fever [20]. However, unlike that study, we did not find that cough was a predictive symptom for influenza. Possible reasons for such a difference include the potentially different aetiologies for non-influenza cases in the tropics and other regions, and also possible differences in influenza presentation by region. It is therefore important to validate these predictive tools in the local setting where they are used.

In the absence of laboratory testing, using our clinical diagnostic model enabled accurate classification of up to 76% of all cases in our cohort (Table 2). Keeping sensitivity at 90%, we were able to achieve a high negative predictive value of 86%, which is useful for clinicians in excluding influenza cases. The positive predictive value, on the other hand, is low due to the substantial overlap in symptoms between influenza and non-influenza cases. The clinical diagnostic model performed significantly better than standard ILI criteria among our subjects with febrile respiratory infections. It can be easily adapted into various tabular or electronic formats for easy use by clinicians. This, if taken together with specific policy and cost evaluations in the local setting, may help guide initiation of anti-viral treatment or isolation measures during an epidemic or pandemic situation while reducing wrong treatment of non-influenza cases to minimize stockpile wastages.

The strengths of our study are its large sample size, high follow-up rate, and high diagnostic ascertainment, with etiological confirmation of all positive influenza cases. There are some limitations to this study, including the natural bias towards febrile symptomatic cases due to the case definition. Influenza cases do present with mild or asymptomatic infection, but these cases will be difficult to identify in a surveillance program and are less severe in clinical outcome. The results should therefore be interpreted in the context of febrile symptomatic infection requiring physician consultation, which capture the more severe and important cases that affect absenteeism.

In addition, this study predominantly considered young male adults. While we felt that there is no evidence that shows any differences in presentation by gender, further studies are required to determine if similarly high diagnostic ascertainment can be

Table 2. Utility of the predictive probability equation as a clinical diagnostic model in this study under 10-fold cross-validation compared with commonly used ILI criteria (for which no cross-validation is needed).

Variable	Sensitivity (%, and 95% CIs)	Specificity (%, and 95% CIs)	PPV (%, and 95% CIs)	NPV (%, and 95% CIs)	Overall accuracy (%, and 95% CIs)
Predictive probability equation, maximising total sensitivity and specificity	65 (58, 72)	69 (62, 75)	43 (39, 47)	85 (83, 87)	68 (64, 71)
Predictive probability equation, maximising accuracy	18 (8, 29)	96 (93, 99)	67 (57, 76)	77 (75, 80)	76 (74, 77)
Predictive probability equation, setting sensitivity to 90%	90 (89, 90)	26 (20, 23)	30 (28, 33)	86 (83, 89)	43 (38, 48)
Fever ≥37.8°C, cough or sore throat	84 (78, 83)	36 (34, 38)	34 (31, 35)	84 (80, 85)	48 (47, 51)
ILI (Fever ≥38.0°C, cough or sore throat)	69 (64, 71)	55 (53, 57)	37 (35, 40)	81 (79, 83)	58 (57, 60)

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achieved in other age groups. Similarly, consultation biases may exist as the military population have medical consultation patterns that differ from the general population. We re-emphasize that diagnostic tools should be developed in the setting where they are used. Other potential biases include presentation biases from cases which rejected recruitment, presentations after recruitment hours which were not included, and losses to follow-up. Recall biases may exist as we obtained final clinical history two weeks after enrolment into the study, which we felt struck a balance between the risk of recall bias and the desire to capture comprehensively all symptoms during the illness period.

Different diagnostic scores may need to be developed to account for local FRI aetiologies and socio-cultural-demographic differences, but so doing will rely on well-designed local surveillance programs. The best clinical syndrome to be used for surveillance is a potentially interesting question that may be explored by further related studies.

Use of a predictive equation as a clinical diagnostic model can help better predict influenza than the conventional influenza-like illness definition among young adult military personnel with febrile respiratory illnesses. Until cheap, rapid and reliable point-of-care tests become widely available, clinical scores derived from large cohort studies may be of reasonable clinical utility.

Author Contributions

Conceived and designed the experiments: VJL JY JPL MIC PAT BHT. Performed the experiments: VJL JY CHT JPL WHK EASL JCWL JSWC IH. Analyzed the data: VJL JY ARC CHT JPL IH QHG PMK MIC PAT BHT. Contributed reagents/materials/analysis tools: ARC JPL WHK EASL JCWL JSWC IH KWC PJT SHN BHT. Wrote the paper: VJL JY ARC CHT JPL PMK MIC PAT BHT.

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Chapter Seven

Preparedness and Response to Influenza Epidemics and Pandemics

From the evidence included in the previous chapters, influenza epidemics and pandemics have wide-ranging impacts globally. In response to the threat posed by influenza, and the likelihood of pandemics occurring, many countries have developed influenza pandemic preparedness and response plans, following guidance from WHO (1,2). However, the interventions used in many of these plans are based on limited scientific data, and mathematical modeling studies. This chapter describes some of the key pharmaceutical and public health interventions that have been used in influenza pandemic preparedness and response and are the subject of the subsequent studies, and concludes with a systematic review of the literature on mathematical modeling studies that estimate the effectiveness of these interventions.

Pharmaceutical Interventions

An integral part of preparedness plans, pharmaceutical interventions include: the stockpiling of antiviral drugs and prototype pandemic vaccines; the development of vaccine production capabilities to produce pandemic vaccines; and advanced purchase agreements with pharmaceutical companies to make these drugs or vaccines available when needed. Stockpiling of antiviral drugs in particular is widely adopted in well-resourced nations to prepare for an influenza pandemic. These stockpiles provide population level protection as a partially effective drug may still have a substantial impact in reducing spread or severe infections at the population level.

Antiviral drugs

The most widely used antiviral drugs against influenza currently are the neuraminidase inhibitor class of drugs. The two currently approved drugs in widespread use are oseltamivir and zanamivir, which are effective for the treatment of

seasonal influenza and prophylaxis to prevent infection (3,4). It is also effective against the 2009 H1N1 pandemic strain and H5N1 influenza which is a zoonosis of concern in Asia, Africa, the Middle East, and Europe (5-7). Several other investigational neuraminidase inhibitors, including intravenous or intramuscular peramivir, and inhaled CS-8 and T-705 are currently in advanced development (8). The development of drugs with non-oral and non-respiratory routes of administration such as peramivir (intravenous or intramuscular) and intravenous zanamivir is also important especially for severely ill patients, and the intramuscular formulations can also serve to increase compliance in the outpatient setting (9).

Oseltamivir is easily administered orally and has been the most commonly stockpiled drug among countries and inter-governmental agencies. However, seasonal H1N1 viruses prior to the 2009 pandemic have shown high levels of resistance to oseltamivir, (10) and there is also reduced sensitivity of H5N1 viruses to the drug (11). In particular, seasonal H1N1 viruses with a His274Tyr mutation have over 350-fold loss of susceptibility to oseltamivir (3). If higher or more prolonged doses were required during an epidemic or pandemic, existing stockpiles which were mostly computed based on normal-dose therapy would be rapidly depleted. However, oseltamivir is still effective against the 2009-H1N1 pandemic strains even though there have been individual reports of resistant cases (12-15).

Zanamivir is also being increasingly stockpiled as it remains effective for treatment of and prophylaxis against oseltamivir-resistant strains including variants with His274Tyr or Asn295Ser due to a difference in binding sites (3) on the NA protein which are not affected by the oseltamivir resistant binding-site configurations.

However, the oral inhalation device that delivers the drug may be difficult to use in the young and in the elderly, and may cause bronchospasm in those with pre-existing chronic respiratory conditions. As the Singapore Government had stockpiled larger quantities of oseltamivir compared to zanamivir, the first line drug of choice for influenza was oseltamivir, and consequently oseltamivir was used in all of the studies in this thesis where antivirals were indicated.

Another class of antivirals that are also effective against influenza is the adamantanes, such as amantadine and rimantadine. These drugs were effective during the 1968 Hong Kong pandemic and have been widely used previously. However, recent circulating H3N2, 2009 H1N1 and influenza B viruses are resistant to the adamantine class of antivirals despite the lack of clinical use of these drugs in recent years for influenza treatment (5). Despite this, they have a potential role as combination therapy with neuraminidase inhibitors for treatment of influenza cases when the virus is susceptible to adamantanes, or when the circulating strain is oseltamivir-resistant and zanamivir is medically contra-indicated (16,17). Their limited use for seasonal influenza may also result in increased effectiveness as combination therapy due to possibly less selective pressure towards resistant strains in the future.

Vaccines

The current influenza vaccines in use for seasonal influenza prevention are the trivalent inactivated influenza vaccine which is administered by injection, and the live attenuated influenza vaccine which is administered by nasal spray. These vaccines contain three separate influenza strains – one each from influenza A/H1N1, influenza A/H3N2, and influenza B. The strains selected for each vaccine formulation are those

that are predicted by the WHO to be in circulation in the coming influenza seasons. There are two vaccine formulations released each year – one ahead of the Northern Hemisphere winter season, and the other ahead of the Southern Hemisphere winter season. As the vaccine strains are based on predictions, actual matches to the circulating influenza strains vary from season to season, and consequently the preventive effectiveness of the vaccine is higher for better vaccine matches (18). The tropics pose a substantial challenge in the selection of the Northern or Southern Hemisphere vaccine formulations, and the timing of vaccination, because of the multiple peaks each year and high baseline incidence. There is, however, little evidence in the tropics on the effectiveness of one vaccine formulation over the other or the ideal timing for vaccine. One Brazilian study using data from 1999 to 2007 found that the influenza season in Brazil starts before the Southern Hemisphere winter, and that using the composition and timing of the Northern Hemisphere vaccine may increase protection against influenza from 30% to 65% compared to the Southern Hemisphere vaccine that was usually used (19). More studies using virus data from different tropical regions and across different time periods are required to determine the historical matching of the annual vaccine formulations and timing for vaccination.

New vaccine development programs have also focused on a range of influenza strains with pandemic potential (20). Whole viruses, live attenuated intranasal application, cell culture production systems, antigen sparing techniques and a range of adjuvant are being explored (20). Stockpiling of candidate pandemic vaccines before a pandemic may reduce the overall spread and impact of the pandemic if the stockpiled vaccine strains are reasonably matched to the actual pandemic strains. Thus, countries

have been considering stockpiling H5N1 vaccines against currently circulating H5N1 strains in anticipation that the H5N1 strain would result in a future pandemic (21-23). However, the cost-effectiveness of such a strategy is highly dependent on the efficacy of the vaccine and the matching to the pandemic strain, and the time to the next pandemic due to stockpile costs. This has been shown in an economic evaluation publication that I did in 2009 (24). A vaccine with the ability to generate cross-strain reactivity and prime immunity in individuals before an outbreak is another potential pandemic immunization strategy (20). This will be further discussed in Chapter Eight.

New vaccines with: greater cross-protection against conserved viral regions; vaccine libraries for rapid production of candidate vaccines; better adjuvant and antigen-sparing strategies for greater production capacity; and administrative modes for improved immunogenicity and cross- protection (23,25) are alternative approaches that merit consideration. As many countries currently purchase vaccines from overseas-based manufacturing facilities; supplies may be inadequate during a pandemic. Increasing vaccine manufacturing capacity, vaccine stockpiling and priming with pre-pandemic vaccine may protect communities before actual pandemic vaccines become available.

Addressing bacterial co-infections

Antibiotics should also be considered as part of pandemic preparedness stockpiles (26). In 1918, pandemic victims had a high incidence of bacterial pneumonia with multiple bacterial pathogens (26,27). However, several European pandemic preparedness plans did not include antibiotics and most did not consider the required quantity (28). Stockpiles should cover common locally circulating bacterial infections,

and may include amoxicillin/clavulanate, doxycycline, cephalosporins, fluoroquinolones, macrolides or co-trimoxazole (29). Likewise, routine vaccination against bacterial infections such as *Streptococcus pneumoniae*, *Hemophilus influenzae b*, and *Neisseria meningitidis* for at-risk populations should be considered (29). This will reduce the impact of bacterial infections, including in unvaccinated groups as one study showed, the presence of less pneumococcal infection among adults after introduction of childhood pneumococcal vaccination (30).

Non-Pharmaceutical (Public Health) Interventions

Non-pharmaceutical measures have been widely used to reduce the spread and impact of influenza and other infectious diseases for a long time. Examples include the use of quarantine during the plague epidemic in Europe in the 14th Century, to the combination of different measures used during the 1918 influenza pandemic. These public health measures are important as they work via different mechanisms to pharmaceutical interventions and can be complimentary with synergistic effects in reducing the spread of influenza. At the same time, pharmaceutical interventions may not be available in sufficient quantity in all settings, especially in less resourced areas, or they may not be available in a timely manner. A good example of the latter is the availability of vaccines to a new pandemic strain which could take months to develop. Public health interventions therefore play an important role in reducing the impact of influenza, especially in the absence of anti-viral drugs or vaccines. The following describes some of the common measures that have been used for influenza epidemic and pandemic preparedness and response.

Many population or individual measures have been used to reduce the spread of influenza. Although these measures have been widely used, there has been a lack of scientific evidence for their actual effectiveness before the 2009 pandemic. Much of the existing evidence has been obtained from observational examples from the 1918 pandemic showing the possible effectiveness in early, combined, and sustained measures in reducing influenza spread in an era before the availability of pharmaceutical interventions (31-33).

Personal hygiene measures

Personal hygiene measures are often recommended as part of universal hygiene and infection control, despite a lack of clear evidence in specifically preventing the spread of influenza (34). These measures include hand washing before meals; avoiding hand contact with eyes, nose, or mouth; covering nose and mouth when sneezing or coughing; and seeking early medical treatment (including self-isolation) when ill. A systematic literature review found that frequent hand washing was generally effective in reducing transmission of respiratory viruses (35), and hand washing has been shown to be associated with 6% to 44% reduction in non-specific respiratory infections (36). Hand washing has also been shown to be highly effective in preventing the transmission of SARS, another disease with respiratory transmission (37,38). However, adding virucidal or antiseptic agents to normal hand washing may not increase its effectiveness (35), even though alcohol disinfectants have been shown to inactivate influenza viruses (39). As few of these measures have been rigorously studied during actual influenza epidemics, more research is needed. In addition, personal hygiene measures should be supported by other strategies.

Personal Protective Equipment

The use of personal protective equipment such as masks, gloves, and gowns has also been found to be effective in reducing respiratory virus transmission (35). Specifically, facemasks have been used by healthcare workers and the general population in an attempt to reduce the spread of infection from an infected individual, or to reduce the exposure to and risk of infection of other individuals. One systematic review found that there is some evidence supporting the use of masks in infected individuals to reduce the spread of influenza to other individuals (40). For the prevention of risk of infection, one study in Australia found that the use of respiratory masks, both surgical masks and non-fit tested FFP2 masks (equivalent to N-95 masks), significantly reduced the risk of ILIs (41). A household study in Hong Kong found that the use of facemasks by all household members together with hand hygiene reduced influenza transmission - if initiated within 36 hours after the index case's clinical illness onset (40). However, both studies also mentioned that adherence to use was also an issue. Among the different types of masks, a randomized control trial in Canada showed that there was no significant difference in influenza infection rates among hospital nurses caring for potential influenza patients wearing surgical masks or FFP2 masks (42).

Social distancing

Different forms of social distancing have been used in previous influenza pandemics but their impact remains dependent on multiple factors and is mostly unproven during epidemics. The use of contact tracing and quarantine has been used to contain the spread of different diseases for centuries and is a well-accepted concept. However, it is often difficult to show the effectiveness of these measures in a field setting.

Reducing mass gatherings have also been recommended but there are no studies that have conclusively proven the effectiveness of this measure. School closures as a form of social distancing in a closed setting with high infection rates may be effective if undertaken early, decisively and for prolonged periods (43,44). However, schools have to be closed early and for long periods and contact among children outside of school will have to be similarly reduced, resulting in substantial socio-economic cost.

Travel restriction

Transmission of influenza in aircraft has been previously described (45-47). A recent paper from Australia showed that airline passengers had an increased risk of contracting influenza of 3.6% in they sat within two rows of a symptomatic infected individual, and 7.7% if they were within two seats (48). However, there is little epidemiological evidence to show that international air travel restriction by itself is effective in reducing or delaying the spread of influenza. In addition, screening and quarantining travelers entering international borders, widely used in many ports of entry around the world, did not substantially delay virus introduction in past pandemics (34). Although modeling studies have suggested the potential for border controls to prevent influenza entry into a country (49), studies have shown the low positive predictive value and limited efficacy in detecting febrile passengers early in the course of a pandemic when fever prevalence among travelers entering a country is less than 1% (50,51). Screening of travelers may also be inadequate if performed during the virus incubation period or for subclinical infection, or if the travelers are using medications which reduce febrile symptoms. Screening at entry points is therefore by itself insufficient to prevent the entry of influenza into a country (52). Instead of entry screening, WHO recommends exit screening at ports during the early

pandemic phases (53). However, such measures may not be feasible once the pandemic is well underway, will be disruptive to the flow of people, and will not be 100% sensitive and specific.

Mathematical Models

In the absence of adequate field epidemiological studies, mathematical modeling studies are useful to provide a glimpse of the possible outcomes of different interventions when used individually or in combination. Although models are not a complete reflection of reality, they provide decision makers with additional data to suggest which interventions may be effective. At the same time, effective models require good input data from epidemiological and clinical studies and do not negate the importance of performing field studies. The following study looks at some of these mathematical modeling studies which compare the effectiveness of individual and combination strategies to reduce the spread and impact of influenza in a broad variety of settings. The effect of combining individual measures in different combinations can be observed from such modeling studies. These studies have allowed policy makers to develop their preparedness and response plans with greater confidence in the face of limited scientific evidence.

However, many of these mathematical modeling studies are based on specific geographical areas and make many assumptions about the transmission and spread of the influenza virus, the mixing of individuals within the populations, and the operational aspects of the interventions. Therefore, while they are useful to guide decision making, the interventions must be tested in real-life situations to provide

evidence of their effectiveness. This is the basis for the rest of the studies in this thesis and described in further detail in the subsequent chapters.

Study 5

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Combination strategies for pandemic influenza response - a systematic review of mathematical modeling studies

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Abstract

Background: Individual strategies in pandemic preparedness plans may not reduce the impact of an influenza pandemic.

Methods: We searched modeling publications through PubMed and associated references from 1990 to 30 September 2009. Inclusion criteria were modeling papers quantifying the effectiveness of combination strategies, both pharmaceutical and non-pharmaceutical.

Results: Nineteen modeling papers on combination strategies were selected. Four studies examined combination strategies on a global scale, 14 on single countries, and one on a small community. Stochastic individual-based modeling was used in nine studies, stochastic meta-population modeling in five, and deterministic compartmental modeling in another five. As part of combination strategies, vaccination was explored in eight studies, antiviral prophylaxis and/or treatment in 16, area or household quarantine in eight, case isolation in six, social distancing measures in 10 and air travel restriction in six studies. Two studies suggested a high probability of successful influenza epicenter containment with combination strategies under favorable conditions. During a pandemic, combination strategies delayed spread, reduced overall number of cases, and delayed and reduced peak attack rate more than individual strategies. Combination strategies remained effective at high reproductive numbers compared with single strategy. Global cooperative strategies, including redistribution of antiviral drugs, were effective in reducing the global impact and attack rates of pandemic influenza.

Conclusion: Combination strategies increase the effectiveness of individual strategies. They include pharmaceutical (antiviral agents, antibiotics and vaccines) and non-pharmaceutical interventions (case isolation, quarantine, personal hygiene measures, social distancing and travel restriction). Local epidemiological and modeling studies are needed to validate efficacy and feasibility.

Background

Many countries have developed pandemic preparedness plans in response to the threat from pandemic influenza [1], to attempt containment of the virus or to reduce the pandemic's impact. The influenza A (H1N1-2009) pandemic has underscored the importance of such plans, with the World Health Organization (WHO) calling for the activation of pandemic plans worldwide [2]. Although the WHO has made public guidelines for developing pandemic plans [3], the comprehensiveness and standards of pandemic plans differ widely across different countries and continents [4-6]. To ensure the success of these plans, it is necessary to adopt a combination of different strategies.

Although there are existing historical data on the possible success of strategies used in previous pandemics such as personal hygiene, school and workplace closures, and social distancing, these are often anecdotal and difficult to interpret [7,8]. Mathematical models provide a platform for the assessment of multiple interventions in an environment where individual parameters can be altered. The recent increase in mathematical modeling studies on pandemic interventions suggests the effectiveness of these strategies and provides guidance for policy makers. Although the 2009 pandemic has spread rapidly, these combination strategies can be applied in populations yet to be severely affected, for the second wave, or for the next pandemic [9,10]. This systematic review aims to determine the individual components that constitute combination strategies, and the quantitative impact of these combination strategies in reducing pandemic spread and morbidity.

Methods

This study explored available mathematical modeling publications on the effectiveness of combination strategies for an influenza pandemic. To obtain papers on the effectiveness of combination strategies, data for this review were identified by the authors through searches of the PubMed search engine for English language articles and articles translated into the English language. The authors used the following search terms to focus on modeling studies, and those which had a focus on pandemic preparedness and strategies - *influenza* and *pandemic* and (*preparedness* or *strateg** or *model**); *influenza* and *modeling* or *modelling*. The search included all published articles listed on PubMed from 1990 to 30 September 2009 - there were few articles on influenza pandemic planning or modeling before this period.

Abstracts were reviewed where available to determine if a study met the inclusion criteria and the full manuscript was obtained for further scrutiny. Snowball searches by hand were performed on the reference lists of articles meeting the inclusion criteria to find additional studies.

The inclusion criteria were primary mathematical modeling papers that compared and reported the quantitative effectiveness of combination strategies (two or more strategies used together) versus individual strategies for human pandemic influenza. Mathematical modeling papers were those which used quantitative predictive methods to determine the likely impact of strategies, and had descriptions of these methods which could be reproduced or verified. All influenza preparedness strategies were considered, including pharmaceutical and non-pharmaceutical public health strategies. These articles would allow clear comparison on the advantages of combination strategies over and above the impact of individual strategies. An explanation of some of the key strategies are found in the appendix.

Mathematical modeling articles that described the effectiveness of multiple singular strategies but did not analyze the quantitative effect of combination strategies were excluded. Articles that referred to general pandemic preparedness without quantitative evidence, or provided only qualitative discussion were also excluded. Reviews without primary data, articles in abstracts without full publication, and unpublished studies were excluded as their methodology and results could not be verified.

Mathematical models are based on input variables which are assumptions made based on available evidence in specific scenarios. One important assumption is the reproductive number (Ro), which is the average number of secondary infections generated by a single case in a completely susceptible population. No attempt was made in this review to homogenize data across studies for comparison; on the contrary, the heterogeneity of data provides public health professionals with evidence of the effectiveness of strategies across a wide range of assumptions and scenarios. We have instead listed the different types of models used, and the scenarios, interventions, and countries where they were applied.

Results

The search yielded a total of 1,920 papers including overlaps. Of these, 162 used mathematical modeling techniques and on closer review, 144 were excluded based on the exclusion criteria listed in Methods. The remaining 18 studies were included for analysis, together with one additional study identified from the snowball searches (Figure 1). The selected modeling papers that show the effectiveness of combination strategies in increasing the impact of individual strategies are listed in Additional files 1 and 2[11]. The following sections highlight key findings on

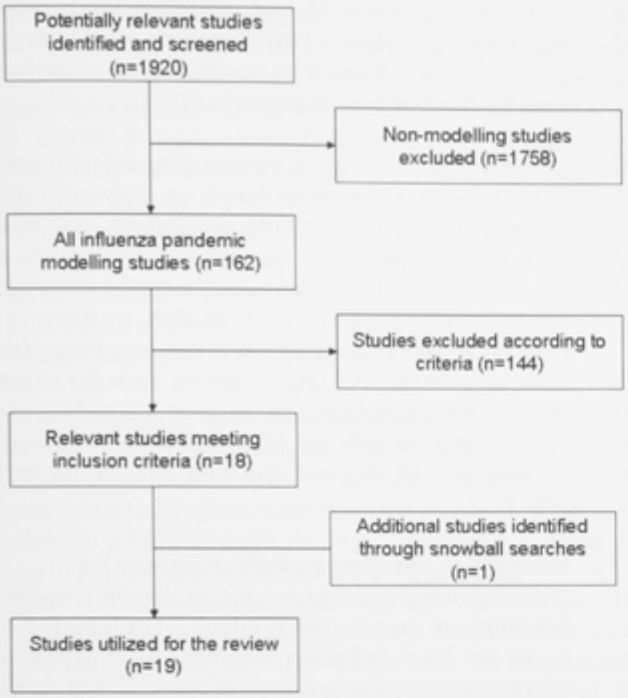

Figure 1
Flow diagram for selection of combination strategy modeling studies.

the effectiveness of combination strategies in these modeling studies on pandemic influenza.

Source containment

Zoonotic influenza such as H5N1 influenza is endemic in several countries, and there is interest in containing a highly virulent pandemic at the earliest sign of localized efficient human-to-human transmission. Two key modeling studies suggested a high probability of success for rapid containment of an influenza epicenter with combination strategies under favorable conditions [9,10]. These studies formed the basis for the epicenter containment strategies recommended by the WHO. Longini showed that antiviral prophylaxis alone could contain a pandemic influenza virus with reproductive number (Ro) less than 1.7; while 70% household quarantine alone was effective up to Ro of 1.7. A combination of quarantine and antiviral prophylaxis was effective up to Ro of 2.1; while a combination of pre-pandemic vaccination, household quarantine and antiviral prophylaxis was effective for Ro of 2.4 [9]. Ferguson found that antiviral prophylaxis for contacts only would have a 90% chance of containing a virus with a Ro less than 1.25, while antiviral prophylaxis for contacts and all individuals in a 10 km zone would have a 90% chance with Ro less than 1.7 [10]. Combined anti-viral prophylaxis and either school and workplace closures or area quarantine provided a similar chance of containment with Ro of 1.7 to 1.8, while a combination of all three strategies would contain a virus with Ro of 1.9 and allow for greater initial surveillance errors [10].

Reducing pandemic spread

Combination strategies can be used to reduce the global spread of the influenza virus [12,13]. Redistribution of limited antiviral drugs can help contain pandemics or reduce the global attack rate (AR) [12]. If global antiviral stockpiles are limited, non-cooperative strategies where countries keep their antiviral stockpiles for their own use can only contain a pandemic influenza virus with Ro less than 1.5; in contrast, if redistribution of 25% of stockpiles from countries that have them to countries that do not, a pandemic with Ro up to 1.9 may be contained, and overall AR reduced by 25% at higher Ro [12].

Another example of combination strategy is reduction of pandemic spread through air travel. Suspension of 99.9% of air travel can only delay individual national epidemics by up to four months, while a combination of local strategies reducing influenza transmission by 40% can delay pandemic spread by up to 10 months [13]. A combination of vaccination and travel restrictions may delay epidemic growth, allowing vaccination of susceptible individuals [14]. With a pandemic starting in July in Asia, the number of United States (US) metropolitan cases was 102.4 million - 0.1% daily vaccination alone reduced this to 73.0 million, and vaccination together with travel restriction reduced this to 56.9 million [14].

Combination strategies may have substantial impact in reducing the global spread of resistant viruses. For example, if the probability of emergence of anti-viral drug resistance was 1%, antiviral monotherapy was associated with overall AR of 67% and resistant AR (RAR) of 38% [15]. In contrast, early combination chemotherapy was associated with reduced AR of 58% and RAR of 2%, while sequential multi-drug chemotherapy was associated with AR of 57% and RAR of 3%.

Mitigating pandemic impact

During the pandemic, several studies found that combination strategies delayed the spread of the virus, reduced the overall number of cases, and delayed and reduced the peak AR much more than individual strategies which may be ineffective if used alone [16-19].

A study using individual-based modelling in the United Kingdom and United States examined the effects of antiviral treatment and prophylaxis, vaccination, case isolation, household quarantine, school and workplace closure and travel restrictions in pandemics with Ro of 1.7 to 2.0. It found that external or internal travel restrictions alone would delay spread by two to three weeks only if more than 99% effective [16]. Reactive school and work-

place closures alone did not impact on overall AR, but reduced peak AR by about 40%; antiviral treatment and prophylaxis within the household reduced overall AR by 35% and peak AR by 45%.; while household quarantine alone reduced overall AR by 10% and peak AR by 20%. Combination antiviral treatment and prophylaxis, and household quarantine reduced overall AR by 40% and peak AR by 60%. Combination school and workplace closure, antiviral treatment and prophylaxis, and household quarantine reduced overall AR by more than 60% and peak AR by more than 80%. Combination antiviral treatment and prophylaxis, school closure and 20% pre-pandemic vaccination reduced overall AR by more than 60% and peak AR by more than 75%. Combination antiviral treatment and prophylaxis, household quarantine, school and workplace closure, and effective border control reduced overall AR by more than 70% and peak AR by more than 90% [16].

Similarly, another individual-based stochastic simulation model in Chicago evaluating the effects of antiviral treatment and prophylaxis, quarantine, isolation, school closure, community and workplace social distancing showed that social distancing alone may reduce overall AR by 60% for pandemic Ro of 1.9 but combination antiviral treatment and prophylaxis, quarantine, social distancing, and school closure could reduce overall AR by more than 90% for similar pandemic Ro of 1.9 [17].

Another study in France examined the effects of antiviral treatment and household prophylaxis, vaccination, household quarantine, school and workplace closure at the individual and community level [20]. Treatment only with anti-viral drugs did not affect AR substantially. Antiviral prophylaxis of 90% of household contacts reduced AR by 50%. Vaccination of 70% of the population within one day reduced AR by 80%. A combination of antiviral treatment and prophylaxis, and household quarantine reduced AR by 90% [20].

An Australian individual-based stochastic simulation model assessed the effects of non-pharmacological pandemic mitigation measures of case isolation, school closure, workplace non-attendance and community contact reduction [21]. For a pandemic with Ro of two, school closures alone reduced AR by 20%, case isolation by 40%, workplace non-attendance by 15%, and social distancing by 25%. In contrast, combination of all these measures reduced AR by more than 95% [21].

A deterministic compartment model using *InfluSim* based on a small community of 100,000 population assessing the effects of antiviral treatment, case isolation and social distancing showed that case isolation and social distancing could reduce overall AR by 25%, and antiviral treatment alone by 20%, compared with a reduction of 40% with a combination of case isolation, social distancing and antiviral treatment [18]. The triple combination strategy could delay the peak by one month compared with 10 days for the first two strategies [18].

Another study using a deterministic model with a stochastic simulation component based on Italy examined the effects of household antiviral prophylaxis, pre-pandemic vaccination, and social distancing via closure of all schools, public offices and public meeting places [22]. In a pandemic with an attack rate of 35%, vaccination alone reduced AR by up to 10% even at vaccine efficacy levels of 70%; antiviral prophylaxis alone for even the entire pandemic duration reduced AR by up to 6% only; and social distancing alone reduced AR by less than 1%. However, a combination of all three measures reduced AR by up to 30% [22].

Intervention effectiveness with changes in Ro

The relative success of interventions depends on the transmissibility of the pandemic, which is commonly reflected in the Ro. In an influenza pandemic with higher Ro, the effectiveness of interventions is reduced and individual interventions are commonly ineffective. However, across most scenarios, combination strategies maintain some effectiveness as shown clearly in the studies on containment by Longini [9] and Ferguson [10].

A stochastic agent-based discrete-time simulation model in the United States examining the effect of antiviral prophylaxis, vaccination, school closure and travel restriction found that for a pandemic influenza virus with Ro of 2.4, unlimited antiviral prophylaxis and best vaccination program may reduce cases by 64% and 34% respectively, while school closure within seven days of pandemic onset may reduce cases by 14%, social distancing within seven days by 6%, and travel restrictions exceeding 90% was ineffective [19]. However, a combination strategy of all of these measures may reduce cases by 99.8% [19]. The effectiveness of any strategy in delaying the pandemic or reducing the AR is highly dependent on the Ro. For example, for a pandemic with Ro of 1.6, individual strategies of prophylaxis, vaccination, or school closures had very high effectiveness [19]. However, once the Ro increased beyond 2.0 (which is similar to the Ro for the 1918 pandemic), individual strategies were much less effective, whereas combination strategies still maintained effectiveness across a range of Ro.

An individual-based model in Italy assessing the effects of household, school and workplace antiviral prophylaxis, vaccination, international air travel restriction, social distancing via school closure and closure of some public offices showed that without any interventions, importation of pandemic influenza would occur 37 to 77 days after the first case elsewhere in the world. Air travel restric-

tion would delay introduction by one week to one month. For a pandemic with Ro of 1.7, travel restriction and social distancing did not affect overall AR, household prophylaxis reduced AR by 50%, and vaccination reduced AR by 0 to 40%. A combination of antiviral prophylaxis, social distancing, vaccination, and travel restriction reduced AR by more than 90% [23]. For a pandemic with Ro of 2.0, travel restriction in fact increased overall AR by 1% and peak AR by 20%. Household prophylaxis reduced AR by 35%, while vaccination reduced AR by 0 to 30%. A combination of antiviral prophylaxis, social distancing, vaccination, and travel restriction reduced AR by 80%.

Disadvantages of individual measures

An individual-based stochastic model in Hong Kong looking at the effects of antiviral prophylaxis, case isolation and household quarantine reported that in a pandemic with Ro of 1.8 and AR of 74%, household quarantine could reduce AR to 49%; household quarantine and isolation to 43%; household quarantine with anti-viral prophylaxis to 44%; household quarantine, isolation and antiviral prophylaxis to 40% which was recommended. Although adding contact tracing and quarantine of all contacts to the latter combination strategy reduced AR to 34%, the number of people under quarantine would be excessive. Therefore, contact tracing was not recommended [24].

Another study examining the effects of antiviral treatment and prophylaxis, home quarantine and social distancing based on a community of a million population with the assumption that pandemic influenza was introduced by an undetected airline passenger, found that if a pandemic Ro was 3.0, individual interventions would result in increased transmission while combination measures may break community transmission [25]. This was similarly shown by Ciofi and colleagues for a pandemic with Ro of 2.0 [23].

A deterministic compartmental model evaluating the effects of antiviral treatment and prophylaxis, vaccination, case isolation and air traffic reduction globally demonstrated that individual strategies such as case isolation and air travel restrictions may result in higher peak AR even though overall AR could be reduced [26].

A study in Taiwan evaluated the effects of enhanced ventilation, use of respiratory mask and vaccination on pandemic influenza transmission in a school [27]. Vaccination alone of 80% of children was effective in preventing the spread of the virus but this was only if a suitable vaccine was available, which is often not the situation. A combination of masks and ventilation, or a combination of vaccination and masks achieved similar effectiveness [27].

Discussion

Many modeling studies were performed as a result of H5N1 influenza threat and an impending pandemic, but all have used parameters based on historical pandemics and existing studies on the influenza transmission. In addition, these studies provided sensitivity analyses across a wide range of influenza parameters. As such, they are directly relevant to the 2009 influenza pandemic which has an Ro of between 1.2 to 1.6 [28], similar to the 1957 and 1968 influenza pandemic [16], and for future pandemics. At the same time, the 2009 influenza pandemic provides the opportunity to study unknown variables to validate and refine these models.

All of these modeling studies in various settings, and using different models and assumptions, consistently show that combination strategies are more effective compared to individual strategies. Given the lack of good experimental, observation or controlled studies on these strategies, and the difficulties of performing trials during a pandemic, it is difficult for policy makers to know the effectiveness of their policies. These modeling studies provide policy makers with a suggestion of the effectiveness of different combination strategies. At the same time, new models will have to be developed using local data to provide realistic outcomes for local settings. The diverse methodology available from these studies provides sufficient information for countries to build and validate their results locally.

Although the use of individual-based and other stochastic models provide better data resolution, deterministic models mentioned in this review show similar outcomes [18,22,23,27]. These deterministic or simple stochastic compartmental models are much easier to build and may provide rapid results for policy making. This is especially true in countries where the vast amounts of data required for individual-based and complex stochastic models may not be available compared with high-income countries where most sophisticated models were built.

The use of combination strategies necessitates the availability of resources and feasibility for each individual component. For example, stockpiling of pharmaceutical agents is an integral part of preparedness plans and currently widely adopted in well-resourced countries. The increase in anti-viral drug resistance underscores the importance of combination drug use and provides policy makers with recommendations for their stockpiles [15]. Combination stockpiles of sufficient amounts of different antiviral drugs such as oseltamivir, zanamivir and adamantanes will allow for early combination chemotherapy or sequential multidrug therapy which was modeled to be effective against antiviral resistance when a small secondary stockpile was used to augment a primary stockpile

[15]. The United States Federal stockpile is composed of 80% oseltamivir and 20% zanamivir, and several million doses of rimantadine from previous stockpiles [29]. The United Kingdom has purchased additional antiviral drugs to ensure it has a total stockpile for 50% of its population, comprising 68% oseltamivir and 32% zanamivir [30]. Bacterial pneumonia results in substantial morbidity and mortality among pandemic influenza cases [31,32]. Antibiotics should therefore be considered for stockpiling [31]. Stockpiles should take into account common locally circulating bacteria, and recommended amounts range from 10 to 25% of the population [33]. In contrast to antiviral drugs that are not widely used, antibiotics can be part of a rolling stockpile which ensures sufficient stockpiles without expiry issues. Vaccination against bacterial infections should likewise be considered.

From the effectiveness of combination strategies in reducing global spread of influenza or resistant viruses [12-15], resource-rich countries should consider redistributing their resources for the greater global benefit and their own benefit if they have yet to be affected by the pandemic. Controlling local outbreaks through combination strategies can reduce global spread, and countries affected early during the pandemic should be provided with assistance [13].

Vaccines are part of many combination strategies and modeling has shown that introduction of a vaccine four months after the pandemic virus has arrived has limited effectiveness, while stockpiling prototype pandemic vaccines could reduce overall AR [16]. Therefore countries were stockpiling H5N1 vaccines as candidate pandemic vaccines [34,35]. However, if the pandemic influenza virus is totally different from the vaccine virus, the vaccines would be of negligible effectiveness. Investments are needed to develop new vaccines with greater cross-protection against conserved viral regions; vaccine libraries to quickly produce candidate vaccines; better adjuvants and antigen-sparing strategies to increase production capacity; and modes of administration for improved immunogenicity and cross- protection [36,37].

Although some individual strategies may seem very effective, they may not be feasible and models assist policy makers in avoiding potentially disastrous decisions. Social distancing has been widely used in epidemics [7] but their impact remains unclear and highly dependent on disease severity, transmission, and risk groups affected. Local interventions such as school closures may be effective if done early, decisively, and for prolonged periods [20,38-40]. A United Kingdom model based on a 1957-like pandemic showed more than 20% case reduction if the Ro were low (<2) and schools were closed early, but less than 10% case reduction in pandemics with high Ro [38]. A French study showed that prolonged closure and limiting contact among children outside school may reduce cases by 17% and peak AR by 45% [39]. However, school closures and limiting social contact may be socio-economically difficult to achieve. Another study found that total closure of schools and workplaces reduced AR by 95%. However, the socio-economic impact would be unimaginable [20]. Similarly, most modeling studies found that travel restrictions alone did not impact overall AR [13,16,19,23]. Reducing air travel has been modeled to be effective in delaying pandemic spread if nearly 100% reduction can be achieved [13,16], and will be difficult if not impossible to achieve [41]. If used alone, local epidemic severity may increase because restriction-induced travel delays can push local outbreaks into high epidemic season [14].

Although combination strategies are more effective than individual measures, not all combination strategies may be feasible. Active surveillance, isolation of cases, and quarantine of close contacts are important interventions during epicenter containment. These interventions may reduce the Ro of the disease to below one and contain the outbreak. However, it is often difficult to ensure total compliance with these measures and if used alone, will result in missed cases due to surveillance failures, isolation facility exposures, and quarantine failures as shown in the SARS experience [42]. A Hong Kong modeling study found that although contact tracing and quarantine of all contacts was effective, it was not feasible because the number of people under quarantine would be excessive [24]. Therefore combination strategies enable policy makers to leverage on the effectiveness of some measures and reduce potential negative impact of others.

For combination strategies to work, they have to be tailored for each scenario at organizational, community, national, and international levels. To facilitate integration of interventions into effective combination strategies, more evidence is needed through targeted research, for example, the effectiveness of non-pharmaceutical interventions (e.g. personnel cohorting, school closures or reduction in air travel). In the absence of definitive studies, mathematical modeling studies provide an effective means of assessing the effectiveness of these strategies.

A limitation of this study is the restriction of our searches to the PubMed database. While we have made attempts to include additional articles from snowball searches, there is the potential for other published or unpublished studies to be missed from other databases and private sources. Other intrinsic limitations of modeling studies exist, and include the fact that they are based on theoretical epidemiology and not fully based on clinical or epidemiological evidence. For example, widespread use of pandemic vaccines raises safety concerns, and widespread use of antiviral drugs raises concern for antiviral resistance. Viral

transmission during treatment with anti-viral drugs is also not well understood. It is therefore important to perform clinical and epidemiological studies during pandemic or seasonal influenza to understand the effectiveness and impact of these interventions. Models are also highly dependent on the assumptions and input variables, and are specific for a local context. However, if these limitations are understood by decision makers, modeling provides a reflection of the possible outcomes, helps to delineate possible strategies for inclusion, and avoids costly errors.

Conclusion

Modeling studies show that combination strategies increase the effectiveness of individual strategies, guard against individual failures, and may reduce socio-economic impact. In the initial phases of an influenza pandemic, combination strategies provide the opportunity to contain the novel virus or delay its spread, allowing unaffected areas within a country and other countries to activate preventive strategies. During a pandemic, combination strategies allow for different strategies to have synergistic effect in reducing the impact of pandemic influenza, and the socio-economic impact of individual interventions. Finally, combination strategies protect against failure of individual interventions and should be considered in preparedness plans.

Abbreviations

AR: Attack rate; RAR: Resistant attack rate; Ro: Reproductive number; US: United States; WHO: World Health Organization.

Competing interests

VJL has received unrelated research support from GlaxoSmithKline. AWS has received honoraria and reimbursements to attend conferences by GlaxoSmithKline, Novartis, and Sanofi Pasteur.

Authors' contributions

VJL contributed to the design, data collection, and manuscript writing. DCL and AWS contributed to the design and manuscript writing of this study.

Appendix

Description of key variables used in the models

Reproductive number (R) - Number of secondary infections generated by a single primary infection. The basic reproductive number (Ro) represents this number when the entire population is susceptible.

Anti-viral treatment - Treatment of individuals infected with influenza. Most of the studies use neuraminidase inhibitors such as oseltamivir as the drug of choice.

Anti-viral prophylaxis - Administration of anti-viral drugs to well contacts to prevent influenza infection. Prophylaxis here refers to post-exposure prophylaxis in a circumscribed area (household, school, workplace, geographical area).

Vaccination - Administration of an influenza vaccine to prevent influenza infections.

Quarantine - Segregation of well individuals exposed to influenza to prevent spread. Area quarantine is segregation of a geographical area with influenza cases within. Household quarantine is segregation of the household where a case has occurred.

Travel restrictions - Reduction in travel (air or border travel) by a quantum mentioned in the text.

Social distancing - Reduction in contact through strategies such as school and workplace closures, travel reductions, reduction in mass gatherings, behavioral changes in reducing contact, as mentioned in the text.

Additional material

Additional file 1

Table S1. *Combination strategy modeling studies to reduce the pandemic spread.*

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[http://www.biomedcentral.com/content/supplementary/1741-7015-7-76-S1.DOC]

Additional file 2

Table S2. *Combination Strategy Modeling Studies to Mitigate the Pandemic Impact.*

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Table S1: Combination strategy modeling studies to reduce the pandemic spread.

Authors / Source	*Simulation Model Type*	*Strategy*	*Country, WHO Pandemic Alert Phase*	*Ro*	*Strategies Compared*	*Outcome Measures*	*Brief Results*
Longini et al, Science, 2005 9	Stochastic individual-based model	Containment of pandemic influenza epicenter	Thailand, 4	1.1 to 2.4	Household, school, workplace antiviral prophylaxis, pre-pandemic vaccination, area quarantine	1) Cases 2) Containment proportion 3) Escapes	○ Quarantine + pre-pandemic vaccination, + anti-viral prophylaxis effective in containing virus of up to Ro = 2.4 compared to ≤1.7 for individual strategies
Ferguson et al, Nature, 2005 10	Stochastic individual-based model	Containment of pandemic influenza epicenter	Thailand, 4	1.1 to 1.9	Geographical antiviral prophylaxis, school and workplace closure for 21 days, area quarantine for 21 days	1) Cases 2) Probability of elimination	○ Blanket anti-viral prophylaxis of entire country will contain virus with Ro<3.6 but not feasible ○ Combined anti-viral prophyalxis + school and workplace closure + area quarantine is 92% effective (95% CI 91% to 97%) at containing virus with Ro = 1.9 and allows for greater surveillance errors, compared to Ro <1.25 to 1.7 for individual measures
Colizza et al, PLOS Med, 2007 11	Stochastic meta-population compartment model	Global cooperative strategies	Global, 4 onwards	1.1 to 2.3	Redistribution of anti-viral stockpiles	1) Cases 2) Days to arrival 3) Days to peak	○ In the event that anti-viral stockpiles are limited, non-cooperative strategies can only contain pandemics with Ro<1.5. ○ Cooperative strategies (where countries redistribute 25% of the drugs to other countries in need) can contain pandemics up to Ro = 1.9 and even at higher Ro = 2.3 reduces overall AR by 25%.
Cooper et al, PLOS Med, 2006 12	Stochastic meta-population compartment model	Air travel versus local measures	Global, 4 onwards	Not applicable	Air travel and local interventions (isolation, behavior change, antiviral use) to reduce influenza transmission	1) Days to peak	○ Even with 99.9% of air travel suspended, epidemics in individual countries would be delayed by 102 days (IQR 61, 133). ○ Reduction in transmission of influenza by 40% using combination of local strategies could delay the pandemic's spread by 262 days (IQR 105, 349).
Epstein et al, PLOS One, 2007 13	Stochastic meta-population compartment model	Air travel and vaccination	United States, 4 onwards	1.4 to 1.7	Travel restrictions and vaccination	1) Cases 2) Days to arrival 3) Days to peak	○ When travel restrictions are imposed with R_0 = 1.7, mean days to arrival of the pandemic increased by two to three weeks if originates in Hong Kong or Sydney but no impact if originates in London. ○ Vaccination-only does not substantially impact FPT but reduces total number of cases by 27 to 81% depending on country of origin. ○ Combination of vaccination and travel restrictions delays arrival by 0 to 5 weeks and reduces cases by 43 to 84%
Wu et al, PLOS Med, 2009 14	Stochastic individual-based multiple-compartment model	Anti-viral resistance	Global, 4 onwards	1.8	Treatment with oseltamivir, zanamivir, and adamantanes	1) Overall attack rate (AR) 2) Resistant attack rate (RAR)	○ At probability of emergence of drug resistance (pA) of 0.1, 40% treatment and Ro = 1.8, monotherapy has AR 72% (95% CI 71%, 73%) and RAR 66% (95% CI 60%, 71%), early combination chemotherapy has AR 63% (95% CI 63%, 63%) and RAR 18% (95% CI 18%, 18%), sequential multi-drug chemotherapy has AR 63% (95% CI 63%, 33%) and RAR 17% (95% CI 15%, 18%).

Table S2: Combination Strategy Modeling Studies to Mitigate the Pandemic Impact.

Authors / Source	Simulation Model Type	Strategy	Country, WHO Pandemic Alert Phase	Ro	Strategies Compared	Outcome Measures	Brief Results
Ferguson et al, Nature, 2006 15	Stochastic individual-based model	Combination of pandemic strategies	United States and Great Britain, 5 & 6	1.4-2.0	External or internal travel restrictions, school and workplace closures until 3 weeks after last detected case, antiviral treatment and household prophylaxis, household quarantine for 14 days	1) Overall AR 2) Peak daily AR 3) Days to peak	○ With Ro of between 1.7 to 2.0, external or internal travel restrictions alone delays spread by 3 to 4 weeks but only if 99% effective. ○ Combination treatment + household, school, work prophylaxis + school closure + effective border controls reduces overall AR by >70% and peak AR by >90%, compared to reduction in AR by <35% and park AR by <45% with individual strategies
Halloran et al, Proc Natl Acad Sci USA, 2008 16	Stochastic individual-based model	Combination of pandemic strategies	Chicago, United States, 5 & 6	1.9-3.0	Antiviral treatment and household prophylaxis, case isolation, quarantine of contacts for 10 days, school closure, workplace and	1) Attack rate	○ Social distancing alone reduced overall AR by 40-65% for Ro=3.0 to 60% for Ro=1.9 ○ Combination of treatment + prophylaxis + case isolation + contact quarantine + school closure + social distancing reduced overall AR of 53-

					community social distancing (closing theaters, reduced visits to restaurants, shops and public locations, banning mass gathering)		85% for virus with Ro=3.0 and >90% for Ro=1.9
Duerr et al, BMC Infect Dis, 2007 17	Deterministic multiple compartment model	Combination of pandemic strategies	Germany, 5 & 6	2.5	Case isolation, anti-viral treatment, social distancing (school and day care center closure, canceling mass gathering events, behavioral changes)	1) Cases 2) Days to peak	○ Case isolation + social distancing + anti-viral treatment delays peak by 1 month and reduces overall AR by 40%, compared to delay for <2 weeks and reduction in AR by 20% with individual strategy
Germann et al, Proc Natl Acad Sci USA, 2006 18	Stochastic individual-based compartment model	Combination of pandemic strategies	United States, 5 & 6	1.6-2.4	Antiviral prophylaxis (household, school, workplace), vaccination, continuous school closure, social distancing (travel restriction, quarantine,	1) Cases 2) Days to peak	○ Travel restrictions are generally ineffective ○ For Ro=1.6, combination of all measures will reduce cases by almost 100% compared to 23% for social distancing to 99% for unlimited prophylaxis or best vaccination program

					behavioral changes)		○ For Ro=2.4, combination of all measures will reduce cases by 99.8% compared to 6.3% for social distancing to 64% for unlimited prophylaxis and 34.2% for best vaccination program
Wu et al, PLOS Med, 2006 23	Stochastic individual-based model	Household-based strategies	Hong Kong, 5 & 6	1.8 (range 1-3)	Contact tracing, case isolation, household antiviral prophylaxis, household quarantine until 7 days from last case	1) Cases 2) Attack rate 3) Days to peak	○ For Ro=1.8, combination of all measures reduce overall attack rate (AR) by 55% compared to 33% with quarantine only
Roberts et al, J R Soc Interface, 2007 24	Stochastic meta-population compartment model	Combination of pandemic strategies	New Zealand, 5 & 6	1.1-3.0	Targeted antiviral treatment and household prophylaxis (TATP), social distancing (school and workplace closure), household quarantine	1) Attack rate 2) Reduction in reproductive number (R)	○ For Ro=2.0, individual interventions would keep effective R between 0.95 to 2.0. Combination strategies would reduce R to between 0.4 to 0.8.
Nuno et al, J R Soc Interface, 2007 42	Stochastic meta-population compartment	Combination of pandemic strategies	US, UK, Netherlands, 5 & 6	1.6-2.4	Transmission control measures (increased personal hygiene, isolation of infected	1) Cases 2) Hospitalizations 3) Deaths	○ Transmission control measures alone reduces infections, hospitalizations and deaths by about 30% ○ Antiviral drugs and vaccines in

	model				individuals), antiviral treatment and prophylaxis, vaccine		sufficient quantities can lead to >99% decrease in outcomes ○ Combination of all 3 measures reduce impact by an additional 40-80% compared to antiviral drugs and vaccines only
Milne et al, PLOS One, 2008 20	Stochastic individual-based model	Combination of non-pharmaceutical strategies	Albany, Australia, 5 & 6	1.5-2.5	School closures, case isolation, workplace non-attendance, community contact reduction	1) Attack rate 2) Peak daily AR	○ Combination of all 4 strategies reduced the overall AR by >90%, compared to 15% to 40% with individual interventions
Flahault et al, Vaccine, 2006 25	Deterministic compartment model	Combination of pandemic strategies	Global, 5 & 6	1.85-3.4	Vaccination, case isolation, antiviral treatment and prophylaxis, reduction in air travel	1) Attack rate	○ Case isolation reduces AR by 9%, air travel restrictions by 1% ○ Addition of treatment to the above reduced AR by additional 10%, while addition of vaccination and treatment reduced AR by additional 60%
Carrat et al, BMC Med, 2006 19	Stochastic individual-based multiple-compartment model	Combination of pandemic strategies	France, 5 & 6	2.07	Vaccination, antiviral treatment and household prophylaxis, quarantine, school and workplace closures	1) Attack rate 2) Days to peak	○ Combination of anti-viral treatment, prophylaxis of household contacts, and household quarantine reduced AR by 83% (range 75% to 99%). ○ Treatment along reduced AR by 7% (range 5-9%), while household

							prophylaxis reduced AR by 23% (20-24%) ○ Only total closure of schools and workplaces was as effective, reducing AR by 79% (61-99%)
Ciofi et al, PLOS One, 2008 22	Deterministic compartment model	Combination of pandemic strategies	Italy, 5 & 6	1.4-2	Vaccination, household antiviral prophylaxis, air travel restrictions, closure of schools and workplaces (non-essential public offices) for 4 weeks starting from 4 weeks after pandemic	1) Attack rate 2) Peak daily AR 3) Days to peak	○ Ro=1.7, air travel restriction of 90% reduced AR by 0%; closure of schools/workplaces by 0%, prophylaxis by 49.9%, vaccination within 1 month by 42.2%. ○ Ro=2.0, air travel restriction of 90% reduced AR by 0%; closure of schools/workplaces by 0%, prophylaxis by 35.7%, vaccination within 1 month by 30.0%. ○ Combination of prophylaxis, school/workplace closures, vaccination, and air travel restrictions reduces AR by >90% for all Ro
Rizzo et al, Epidemiol Infect, 2008 21	Deterministic compartment model	Combination of pandemic strategies	Italy, 5 & 6	1.8 (range 1.6-2,0)	Household antiviral prophylaxis, vaccination, social distancing (closure of	1) Attack rate 2) Avoided cases	○ For pandemic with 35% AR, vaccination reduced AR by 0.8 to 21.9%, prophylaxis by 0.3 to 5.4%, and social distancing by 0.3 to 1.7%

					schools for 3 weeks, public offices for 4 weeks, and public meeting places for 8 weeks) at 2, 4 and 8 weeks after pandemic		○ Combination of all 3 methods reduced AR by 5.0 to 33.6%
Chen and Liao, Epidemiol Infect, 2007 26	Deterministic compartment model	Combination of strategies in schools	Taiwan, 5 & 6	2.8 – 16.9 across different childhood age groups	Wearing of masks, ventilation of schools, vaccination	1) Cases 2) Peak of pandemic	○ Masks and ventilation reduces AR by 25-35% and delays epidemic peak by 10-15 days. ○ Vaccination, combination of masks and ventilation, or combination of vaccination and masks prevented epidemics from occurring

Chapter Eight

Influenza Vaccine Cross-Reactivity

As mentioned in the previous chapter, vaccination is the mainstay of influenza prevention and there is a wide body of evidence showing the effectiveness of influenza vaccination in generating antibodies to the influenza strain it targets. However, one important question is the amount of cross-reactivity an influenza vaccine has against other influenza strains. While there is current interest in the production of a universal influenza vaccine that can provide protection against multiple influenza subtypes (1,2), with the current vaccines it is also of interest to determine if seasonal influenza vaccination can provide possible protection against strains of the same subtype from other influenza seasons. Similarly, this may provide some reflection on the possible impact of stockpiling of candidate pandemic vaccines (for example H5N1 vaccines) in reducing the overall spread and impact of the pandemic if the actual pandemic strains are of the same subtype but not completely matched.

In the context of the 2009 pandemic, the pandemic virus is the same subtype (H1N1) as the seasonal H1N1 strains that have been in circulation since 1977. However, the 2009 H1N1 pandemic virus has antigenic properties that are substantially different from seasonal strains as it contains a combination of genes from the re-assortment of human, avian, and swine influenza viruses (3). The 2009 pandemic virus is therefore more closely related to the earlier generations of the 1918 pandemic H1N1 virus. On the other hand, the recent seasonal H1N1 strains first emerged in 1977 and were less related to the 1918 pandemic virus. As such, a study in the United States found that less than10% of adults and almost no children had any cross-reactive antibodies, but up to a third of the elderly have been shown to have cross-reactive antibodies to the

2009 H1N1 virus which could be due to exposure to older strains related to the 1918 H1N1 strains (4).

The following study provides a good platform to test the possible cross-reactivity of current seasonal H1N1 influenza vaccines against the novel pandemic H1N1 strain, and an older 1918-origin strain. The findings will provide evidence of the importance of seasonal influenza vaccination in possibly providing protection against other strains within the same subtype, even during subsequent pandemics if they contain similar component genes. This is especially important in tropical countries such as Singapore where seasonal influenza vaccination levels have been low (5). It will also lend some support to the concept of stockpiling of pre-pandemic vaccines, ahead of any future availability of universal vaccines.

Study 6

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Inactivated trivalent seasonal influenza vaccine induces limited cross-reactive neutralizing antibody responses against 2009 pandemic and 1934 PR8 H1N1 strains

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ABSTRACT

Background: In June 2009, we conducted a prospective study in Singapore on 51 individuals to determine their serologic responses before and following receipt of the 2009 Southern Hemisphere seasonal influenza vaccine.
Materials and methods: Paired serum samples were obtained before and 3–4 weeks after vaccination. Virus microneutralization assays were performed to quantify antibodies against A/Brisbane/59/2007 vaccine, pandemic H1N1-2009 and A/Puerto Rico/08/34 H1N1 strains.
Results: Post-vaccination, 43%, 12% and 24% of subjects displayed a 4-fold or greater rise in neutralizing antibody titers against the three strains, respectively. There was a positive correlation among individuals who showed increased titers to both pandemic H1N1-2009 and A/Puerto Rico/08/34 ($p < 0.001$). However, this correlation was not observed for A/Brisbane/59/2007 with either strain. The relative conservation and accessibility of predicted B-cell epitopes may explain the limited cross-reactivity of the antibodies directed against common H1N1 epitopes.
Conclusions: These results suggest that seasonal influenza vaccination confers a certain degree of cross-protection to other H1N1 strains. The correlation in cross-reactive antibody titers to A/Puerto Rico/08/34 and pandemic H1N1-2009 implies that previous exposure to pre-1957 H1N1 strains may confer some protection against the 2009 pandemic strain.

1. Introduction

The 2009 influenza pandemic was caused by a novel virus strain with a unique combination of genes. Genomic analysis of the influenza A pandemic H1N1-2009 (pdm H1N1-2009) virus revealed a combination of genes from Eurasian and classical swine lineages, and triple-reassortant viruses that have circulated in swine for the past 10 years [1]. Classical swine H1N1 viruses were first isolated in 1930, and are antigenically highly similar to the reconstructed 1918 Spanish influenza pandemic virus [2]. In the late 1990s, classical swine H1N1 viruses reassorted with human H3N2 and avian influenza viruses, resulting in a triple-reassortant H3N2 virus [3–5]. Subsequent reassortment between this H3N2 virus and classical swine H1N1 virus, resulted in triple-reassortant swine H1N2 viruses [6]. Genes from this triple-reassortant swine H1N2 virus and the Eurasian swine H1N1 virus culminated in the pdm H1N1-2009 virus [1].

Human H1N1 viruses circulated from 1918 until 1957, progressively accumulating mutations resulting in marked antigenic drift [7]. Human H1N1 viruses which reemerged in 1977 were similar to those in the 1950s, and underwent further antigenic evolution [8]. Hence, current seasonal human H1N1 strains exhibit significant antigenic differences from the original 1918 pandemic virus.

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It is not surprising that a third of elderly ≥65 years old had pre-existing cross-reactive antibodies to pdm H1N1-2009 most likely attributed to the latter's classical swine H1N1 genes, compared to <10% for adults and virtually none for children. The high baseline titers found in the elderly suggest that previous exposure to related H1N1 virus strains may have contributed to cross-reactive antibody responses [9,10]. Although seasonal influenza vaccines target seasonal H1N1 strains, there is some evidence that they may generate cross-reactive antibodies against pdm H1N1-2009. A case-cohort analysis comparing seasonal vaccine coverage between pdm H1N1-2009 cases and the general population estimates that seasonal vaccination may be 10% effective in preventing pdm H1N1-2009 influenza [11], while another study showed a more modest effect of 3% [12]. A frequency matched case–control study suggested that seasonal influenza vaccine may protect against severe forms of pandemic illness, with a significantly lower mortality rate among vaccinated cases [13]. Following vaccination with the 2007–2008 or 2008–2009 seasonal influenza vaccine, 12–22% of adults aged 18–64 years, <5% of those 60 years or older, and almost none among those less than 18 years displayed seroconversion to pdm H1N1-2009 [9]. However, questions have been raised as to how much these observations can be attributed to selection bias [14].

The pdm H1N1-2009 strain provides an opportunity to understand antibody cross-reactivity between different influenza strains. This is of particular relevance in view of increasing global use of seasonal influenza vaccines, as well as the possible utility of pre-pandemic vaccination [15,16]. This paper aims to determine serological response and cross-reactivity after administration of the 2009 Southern Hemisphere influenza vaccine, to the vaccine H1N1 strain, as well as cross-reactivity against the pdm H1N1-2009 strain, and the 1934 H1N1 Puerto Rico/08/34 or PR8 strain.

2. Materials and methods

2.1. Study design and cohort

We conducted a prospective study in the Singapore Armed Forces (SAF, a conscript military which enlists all males in Singapore after completion of high school) on a group of 51 individuals who were vaccinated on 5 June 2009. The study was completed by 25 June 2009, soon after the first case of community transmission in Singapore on 18 June 2009 [17], and none of the study subjects or others within the military camp had pandemic influenza during that period. All were vaccinated with the trivalent 2009 Southern Hemisphere influenza vaccine comprising A/Brisbane/59/2007(H1N1)-like virus; A/Brisbane/10/2007(H3N2)-like virus; and B/Brisbane/60/2008-like virus.

Blood samples (5–10 ml each) were taken from each individual just before vaccination was performed, and 3–4 weeks after vaccination to allow for sufficient immunological response. Serum was extracted from the samples on the same day, aliquoted and stored at −80 °C for subsequent testing. A questionnaire was also administered to collect data on demographics, previous influenza vaccinations, and post-vaccination adverse effects. Written informed consent was obtained, and this study was approved by the SAF Joint Medical Committee (Research), and the Australian National University's ethics review board.

2.2. Virus microneutralization assay

Using this assay, the paired serum samples were tested against three representative H1N1 strains. We used a Singapore isolate, A/Singapore/GP101/2009(H1N1) which has 99% identity in the hemagglutinin and neuraminidase to the A/Brisbane/59/2007(H1N1)-like vaccine strain (GenBank accession numbers CY068676–CY068677). The pandemic H1N1-2009 strain A/Singapore/GP2651/2009(H1N1) was isolated in Singapore (GenBank accession numbers CY049640–CY049647). A/Puerto Rico/08/34(H1N1) or PR8 is a commonly propagated virus from the 1918 pandemic lineage. Influenza virus strains were propagated in embryonated chicken eggs or Madin–Darby canine kidney (MDCK) cells. The standard microneutralization assay was performed in MDCK cells which were seeded into 96-well plates and incubated at 37 °C and 5% CO_2 for 24 h. Sera were heat-inactivated at 56 °C for 30 min, and 2-fold serial dilutions made in Eagle's minimum essential medium (MEM) starting with the 1:8 dilution. Equal volumes of virus (100 $TCID_{50}$) were incubated with diluted serum samples at 35 °C and 5% CO_2 for 2 h. The cells were washed thrice, and serum-free MEM containing TPCK-trypsin (3 μg/ml) was added. The virus–serum mixtures (50 μl each) were inoculated into the cell monolayers, and incubated at 35 °C and 5% CO_2 for 72 h. The neutralizing titer was defined as the reciprocal of the highest dilution of serum at which the infectivity of 100 $TCID_{50}$ of the virus for MDCK cells was completely neutralized in 50% of the wells. For quality control, neutralization tests were repeated for several selected pairs of serum samples, and the results remained consistent.

2.3. B-cell epitope prediction and homology modelling

B-cell epitope prediction was performed in an attempt to explain why antibodies raised against one strain of H1N1 virus may be able to neutralize a different H1N1 strain. Out of three different prediction servers (BCPred, BepiPred and ABCPred), BCPred was selected to predict B-cell epitopes of the hemagglutinin (HA), neuraminidase (NA) and matrix 1 (M1) proteins across the three strains of H1N1 [18–20]. The locations of the highly conserved 20-mer epitopes predicted by BCPred were mapped in the models of the corresponding proteins of A/California/04/09 strain of pdm H1N1-2009. The amino acid sequences of the components of A/California/04/09 were entered into BLASTP to retrieve the sequences of proteins with homologies of at least 40%, and the PDB and FASTA files of similar sequences were then obtained from the RCSB Protein Data Bank (PDB). MODELLER was used for homology or comparative modelling of three-dimensional protein structures, and automatically calculates a model containing all non-hydrogen atoms [21].

2.4. Statistical analyses

We investigated possible cross-reactivity of antibodies by correlating pre-vaccination antibody titers to different strains, and the strength of the antibody response to different strains following immunological challenge with the seasonal influenza vaccine. We defined the log of the relative increase in antibody titers to each strain as the main outcome of interest. Titers <8 were assigned a value of 4. The relative increase was computed by dividing the post-vaccination titer for each subject by the pre-vaccination titer. We then used linear regression to investigate if the log of the relative increase in antibody titers to each of the H1N1 strains was associated with the log of the relative increase in antibody titers, as well as the log of pre-vaccination antibody titers to either of the other two strains. We also used linear regression to investigate if there was any correlation in pre-vaccination and post-vaccination antibody titers on a log scale. All statistical analyses were performed with STATA 10.0 for Windows (STATACORP, College Park, TX) with the level of significance set at 5%.

Table 1
Post-vaccination change in virus neutralization antibody titers to three influenza viruses, stratified by pre-vaccination titer.

	No. of subjects	Change in titer		No. with post-vaccination titers ≥32 (%)	Geometric mean titer[a]		
		No. with ≥2-fold increase (%)	No. with ≥4-fold increase (%)		Pre-vaccination (95% CI)	Post-vaccination (95% CI)	p-Value
A/Brisbane/59/07 H1N1							
Pre-vaccination titers < 32	0	–	–	–	–	–	–
Pre-vaccination titers ≥ 32	50	39 (78%)	22 (44%)	50 (100%)	100 (85,118)	302 (226,405)	<0.001
All subjects	50	39 (78%)	22 (44%)	50 (100%)	100 (85,118)	302 (226,405)	<0.001
A/Puerto Rico/08/34 H1N1							
Pre-vaccination titers < 32	36	25 (69%)	12 (33%)	13 (36%)	5 (5,7)	14 (10,21)	<0.001
Pre-vaccination titers ≥ 32	15	5 (33%)	0 (0%)	15 (100%)	38 (33,45)	49 (40,58)	0.104
All subjects	51	30 (59%)	12 (24%)	28 (55%)	10 (7,13)	20 (15,28)	<0.001
Pandemic H1N1-2009							
Pre-vaccination titers < 32	42	26 (62%)	6 (14%)	9 (21%)	8 (7,9)	15 (11,19)	<0.001
Pre-vaccination titers ≥ 32	9	3 (33%)	0 (0%)	8 (89%)	51 (34,76)	51 (34,76)	1.000
All subjects	51	29 (57%)	6 (12%)	17 (33%)	11 (8,14)	18 (14,24)	<0.001

[a] Nth root of the product of n numbers where n is the sample size; p-values by paired *t*-test for difference between pre- and post-vaccination titers on geometric scale.

3. Results

Of the 55 personnel initially recruited in the study, 51 (92.7%) provided both pre- and post-vaccination samples. The remaining four dropouts did not complete the study as they were subsequently sent on overseas courses or had since left the military. One subject did not have post-vaccination A/Brisbane/59/2007 titers due to insufficient sample for testing. The average age was 23.75 (inter-quartile range of 20–28, range of 19–46 years). Only 5 (9.8%) had a history of previous seasonal influenza vaccination. None of the participants reported any severe adverse effects after vaccination.

3.1. Cross-reactive neutralizing antibody responses prior to and after seasonal vaccination

The pre- and post-vaccination titer distributions are shown in Fig. 1. Following vaccination with the trivalent inactivated seasonal influenza vaccine, 44%, 24% and 12% of vaccinees displayed a ≥4-fold rise in neutralizing antibody titers against the A/Brisbane/59/2007, pdm H1N1-2009 and PR8 strains, respectively (Table 1). The ratio of the geometric mean titers (GMT) post-vaccination compared to pre-vaccination was about 3-fold for A/Brisbane/59/2007, while that of pdm H1N1-2009 and PR8 was about 2-fold. Upon stratified analysis, the rise in antibody titers against the pdm H1N1-2009 and PR8 strains occurred in those subjects without high pre-vaccination titers (<32), in concordance with previous work showing that further increase in titers is less likely to be observed in individuals who already had high pre-vaccination titers [22].

3.2. Correlation of the neutralizing antibody titers against the 3 H1N1 viruses

Table 2 correlates the relative increase in antibody titers between the different H1N1 strains. In line with our findings in Table 1, post-vaccination increase in titers was negatively associated with higher pre-vaccination titers to the same strain, but not to other strains. However, subjects who showed increased antibody titers against PR8 had a significant and positive correlation to increased titers against pdm H1N1-2009 ($p < 0.001$). Pre-vaccination antibody titers to PR8 and pdm H1N1-2009 were also positively correlated, as were post-vaccination antibody titers. This apparent cross-reactivity between antibodies to PR8 and pdm H1N1-2009, but lack of cross-reactivity between antibodies to pdm H1N1-2009 and A/Brisbane/59/2007, is illustrated graphically in Fig. 2. Subjects with high pre-vaccination titers to A/Brisbane/59/2007 demonstrated a wide range of titers to pdm H1N1-2009; in contrast, individuals with high pre-vaccination titers to PR8 were more likely to also exhibit high pre-vaccination titers to pdm H1N1-2009 than those with low pre-vaccination titers to PR8 (Fig. 2A and B). Furthermore, we observed a much stronger correlation of increase in titers between PR8 with pdm H1N1-2009, as compared to A/Brisbane/59/2007 with pdm H1N1-2009, as well as a positive correlation between post-vaccination titers to PR8 and to pdm H1N1-2009 (Fig. 2C–F). Post-vaccination, 13 subjects (25.5%) showed high neutralizing antibody titers (≥32) to both strains.

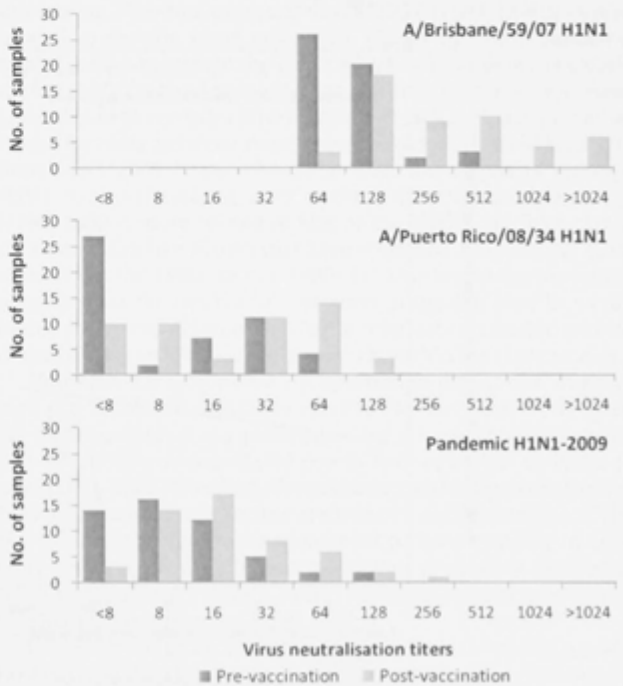

Fig. 1. Distribution of virus neutralization titers in pre- and post-vaccination sera for (A) A/Brisbane/59/08 H1N1, (B) A/Puerto Rico/08/34 H1N1 and (C) Pandemic H1N1-2009. No. of samples is 51 except for the post-vaccination sera for A/Brisbane/59/08 H1N1 where 50 samples were tested.

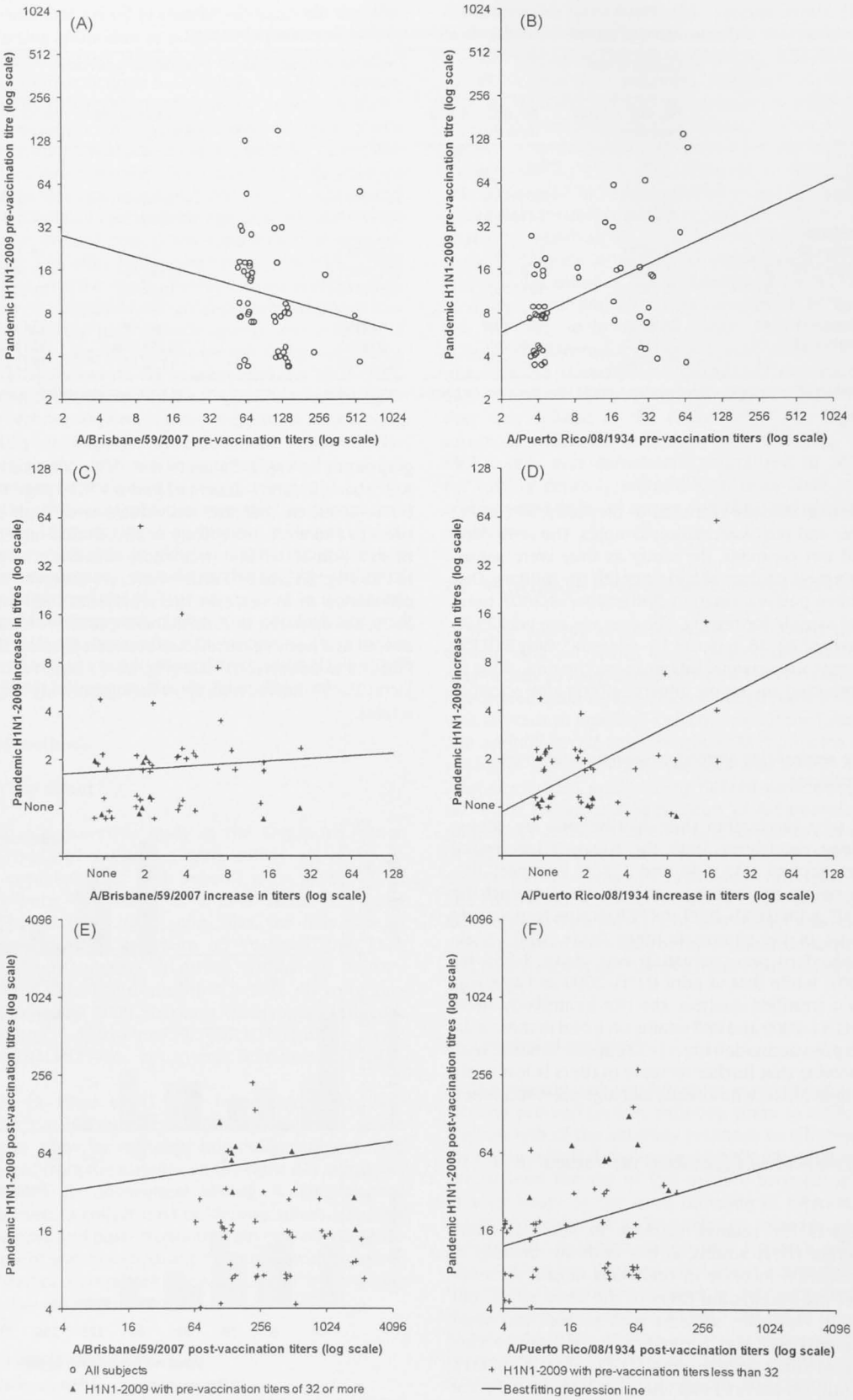

Fig. 2. Correlation of increase in antibody titers among (A) A/Brisbane/59/08 H1N1, (B) A/Puerto Rico/08/34 H1N1 and (C) Pandemic H1N1-2009. Relative increase in titres shown on a log scale.

Table 2
Correlation between log of relative increase in antibody titers, log of pre-vaccination titers, and between log of pre-vaccination and post-vaccination titers for different strains.

	N	R^2	Coefficient	p-Value
Pre-vaccination titers vs increase in titers				
A/Brisbane/59/2007 H1N1 vs A/Brisbane/59/2007 H1N1[a]	50	0.027	−0.274	0.250
A/Brisbane/59/2007 H1N1 vs A/Puerto Rico/08/1934 H1N1	51	0.005	−0.107	0.629
A/Brisbane/59/2007 H1N1 vs Pandemic H1N1-2009	51	0.001	−0.035	0.857
A/Puerto Rico/08/1934 H1N1 vs A/Brisbane/59/2007 H1N1	50	0.023	−0.142	0.288
A/Puerto Rico/08/1934 H1N1 vs A/Puerto Rico/08/1934 H1N1[a]	51	0.078	−0.237	0.048
A/Puerto Rico/08/1934 H1N1 vs Pandemic H1N1-2009	51	0.056	−0.177	0.093
Pandemic H1N1-2009 vs A/Brisbane/59/2007 H1N1	50	0.000	−0.014	0.926
Pandemic H1N1-2009 vs A/Puerto Rico/08/1934 H1N1	51	0.046	−0.205	0.133
Pandemic H1N1-2009 vs Pandemic H1N1-2009[a]	51	0.119	−0.290	0.013
Pre-vaccination titers vs pre-vaccination titers				
A/Brisbane/59/2007 H1N1 vs A/Puerto Rico/08/1934 H1N1	51	0.032	0.325	0.207
A/Brisbane/59/2007 H1N1 vs Pandemic H1N1-2009	51	0.023	−0.245	0.285
A/Puerto Rico/08/1934 H1N1 vs Pandemic H1N1-2009	51	0.194	0.390	0.001
Increase in titers vs increase in titers				
A/Brisbane/59/2007 H1N1 vs A/Puerto Rico/08/1934 H1N1	50	0.052	0.213	0.110
A/Brisbane/59/2007 H1N1 vs Pandemic H1N1-2009	50	0.004	0.052	0.658
A/Puerto Rico/08/1934 H1N1 vs Pandemic H1N1-2009	51	0.246	0.436	<0.001
Post-vaccination titers vs post-vaccination titers				
A/Brisbane/59/2007 H1N1 vs A/Puerto Rico/08/1934 H1N1	50	0.009	0.104	0.521
A/Brisbane/59/2007 H1N1 vs Pandemic H1N1-2009	50	0.020	−0.127	0.332
A/Puerto Rico/08/1934 H1N1 vs Pandemic H1N1-2009	51	0.102	0.258	0.023

[a] Pre-vaccination titer vs increase in titer for the same strain.

3.3. B-cell epitope prediction and homology modelling reveal consensus and potentially accessible epitopes

Using B-cell epitope prediction, the predicted M1 epitope was identical across all three H1N1 strains. Although a few amino acids in the HA and NA epitopes were different among the three strains, the differences involved amino acids with similar properties. Hence, the epitopes predicted for HA, M1 and M2 are relatively conserved across the three strains, while homology modelling revealed their locations on potentially accessible regions. The relative conservation and accessibility of the predicted B-cell epitopes may explain the limited cross-reactivity of the antibodies directed against common H1N1 epitopes.

4. Discussion

In our healthy young adult study population, 78% showed a 2-fold or greater rise in antibody titer to the A/Brisbane/59/2007 vaccine strain. This is consistent with other seasonal vaccine studies using microneutralization with live virus where ~60% have increased antibody titers [23]. We found that our young military population had high pre-existing antibody titers to the seasonal influenza strain, indicating likely exposure to seasonal viruses. We also observed that those with higher pre-vaccination titers to pdm H1N1-2009 and PR8 strains were less likely to have a multiple-fold rise in post-vaccination antibody titers to the same strain, as has been reported in vaccine efficacy studies [22].

Following seasonal influenza vaccination, 12% and 24% of vaccinees showed 4-fold or greater rise in antibody titer to the pdm H1N1-2009 and PR8 strains, respectively. There is currently debate regarding the effectiveness of seasonal influenza vaccination in providing protection against the 2009 pandemic strains, with different studies reporting varying results [11–14]. On the basis of neutralizing antibodies serving as surrogate markers of host protection, our study indicates that seasonal influenza immunization may confer some degree of cross-protection, albeit much lower than the matched vaccine strain. Another study documented similar results, with 78% of adults aged 18–40 years who were immunized with the 2008/09 seasonal influenza vaccine demonstrating seroconversion against the vaccine strain, compared with 12% of vaccinees who revealed seroconversion against A/California/05/2009 [9].

Moreover, by comparing pre-vaccination antibody titers together with rise in titers between three temporally and geographically separate H1N1 strains, our study provides additional insights into cross-protection between influenza strains. There was a statistically significant positive correlation in pre- and post-vaccination antibody levels and rise in antibody levels between PR8 and pdm H1N1-2009, whereas no statistically significant relationship was observed between A/Brisbane/59/2007 and pdm H1N1-2009. The pdm H1N1-2009 strain is thought to be more similar to pre-1957 H1N1 strains, and we have found a significant correlation in antibody titer increases between pdm H1N1-2009 and PR8 to support this notion. Previous exposure to pre-1957 H1N1 strains is postulated to provide some protection against the 2009 pandemic strains, and may explain the lower attack rates among the elderly during the 2009 pandemic [24]. The evidence presented for cross-reactivity between pdm H1N1-2009 and PR8, and lack of similar cross-reactivity between more recent seasonal H1N1 strains and pdm H1N1-2009 is also consistent with the origins of seasonal H1N1 strains circulating prior to the 2009 pandemic. Pandemic H1N1-2009 is more related to PR8, as the origins are from classical swine influenza viruses that have remained antigenically quite stable from the 1930s to late 1990s [1]. Our homology modelling suggests that the mechanism for cross-protection may lie in the highly conserved epitopes located in relatively accessible regions of the hemagglutinin and other proteins within the three strains.

However, the reasons for a proportion of individuals showing high pre-vaccination antibody titers to PR8 and pdm H1N1-2009 are unclear, since all our participants were born after 1957. Some individuals also demonstrated rise in antibody titers to PR8 and pdm H1N1-2009 following administration of the seasonal vaccine. This observation lends further credence to the relatedness of the two strains. Another explanation could be host diversity in antibody responses, with different individuals generating antibodies that can react with different strains following natural infection or vaccination with seasonal H1N1 strains.

Indeed, cross-protection of seasonal influenza vaccine against the H5N1 subtype has also been demonstrated in mice, resulting in reduced virus titer and increased survival [25]. This is attributed to humoral immunity elicited by the N1 neuraminidase component of human H1N1 viruses [26]. A prospective study during the 1957 pandemic (during which a shift from subtype H1N1 to H2N2 occurred)

suggested that accumulated immunity from previous influenza infections contributed to reduced infection rates [27]. From our study, the post-vaccination correlation in antibody titers between PR8 and pdm H1N1-2009 raises the intriguing prospect of the effectiveness of vaccines incorporating historical influenza strains for generating more broad-based antibody responses against future influenza strains. Such vaccines would be particularly useful when antigenic drift leads to new influenza viruses similar to historical influenza viruses, or when a pandemic strain more related to historical influenza viruses appears, as was the case with pdm H1N1-2009.

Limitations of our study include the small sample size available, and the limited age group which were due to the lack of individuals who sought seasonal vaccination just before the 2009 pandemic wave in Singapore. Additional studies should be performed in various age groups and settings to validate these results. Moreover, clinical studies should be conducted to assess the effectiveness of vaccination in preventing actual infection due to different viral strains. More studies are needed to investigate the actual quantum of protection provided by previous influenza exposure, the cross-protection against various influenza strains following natural infection and influenza vaccination, and the clinical efficacy and durability of cross-reactive antibody responses in preventing infection and/or ameliorating disease.

5. Conclusions

Our study has shown that seasonal influenza vaccination generates cross-reactive antibodies against the 2009 pandemic and 1934 PR8 H1N1 strains. Neutralizing antibody responses were positively correlated between these two strains. Bioinformatic analyses of H1N1 hemagglutinin and other proteins may explain the phenomenon of limited antibody cross-reactivity.

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Chapter Nine

Effectiveness of Oseltamivir Ring Prophylaxis

Oseltamivir prophylaxis is another key intervention in influenza preparedness and response plans, and has been shown to be effective in preventing influenza infection in individuals while on the anti-viral drug (1,2). One proposed use of oseltamivir prophylaxis is in rapid containment of an epicenter of novel influenza in combination with other public health strategies (3,4). These two modeling studies suggested the possibility of success of rapid containment to prevent the spread of a novel influenza virus from its origins under favorable conditions of early detection and a low reproductive number (R_0). Rapid containment would involve a combination of pharmaceutical and public health measures including the use of oseltamivir ring prophylaxis within the boundaries of the containment area to reduce the spread of influenza. In spite of the many pandemic preparedness and response plans that adopted the concept of rapid containment, including WHO guidance (5), there is no epidemiological evidence on the effectiveness of such a strategy in real life.

However, rapid containment was not a feasible strategy to prevent the spread of the 2009 pandemic due to the late detection of the epidemic in Mexico, in which the geographical extent of initial spread was too large to make any attempt at containment reasonable. At the same time, the principles of rapid containment could be applied to situations other than preventing the spread of novel influenza from its origins. In other settings where spread of influenza beyond an initial outbreak nidus is undesirable, and where the continuation of activities within the outbreak area is required, rapid containment principles could be applied. This would include outbreaks within essential service facilities such as militaries, hospitals, and civil-defenses; and also in schools and educational institutions to prevent long-term absenteeism. In such situations, other possible measures to reduce spread such as individual-level

quarantine were not feasible as they would hinder the activities and performance of the group. Ring prophylaxis with oseltamivir provides an ideal solution as it would prevent the spread of influenza within the geographically circumscribed area while still allowing for the continuation of activities. At the same time, it will have to be combined with early identification of cases for isolation, and social distancing to prevent the spread of influenza beyond the prophylaxis area.

The following study tests this hypothesis in the first documented evidence of ring prophylaxis to reduce the spread of an influenza outbreak, while preventing absenteeism. It shows the use of ring prophylaxis in four separate outbreaks in the Singapore military which were performed as part of the pandemic preparedness and response strategy. This study conclusively shows the effectiveness of oseltamivir ring prophylaxis as a strategy to reduce the impact of influenza outbreaks in similar settings.

Study 7

Lee VJ, Yap J, Cook AR, Chen MI, Tay J, Tan BH, Loh JP, Chew SW, Koh WH, Lin R, Lin C, Lee CWH, Sung WK, Wong CW, Hibberd ML, Kang KL, Seet B, Tambyah PA. Oseltamivir ring prophylaxis for containment of Influenza A (H1N1-2009) outbreaks. NEJM. 2010; 362:2166-74.

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ORIGINAL ARTICLE

Oseltamivir Ring Prophylaxis for Containment of 2009 H1N1 Influenza Outbreaks

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ABSTRACT

BACKGROUND

From June 22 through June 25, 2009, four outbreaks of infection with the pandemic influenza A (H1N1) virus occurred in Singapore military camps. We report the efficacy of ring chemoprophylaxis (geographically targeted containment by means of prophylaxis) with oseltamivir to control outbreaks of 2009 H1N1 influenza in semiclosed environments.

METHODS

All personnel with suspected infection were tested and clinically isolated if infection was confirmed. In addition, we administered postexposure ring chemoprophylaxis with oseltamivir and segregated the affected military units to contain the spread of the virus. All personnel were screened three times weekly both for virologic infection, by means of nasopharyngeal swabs and reverse-transcriptase–polymerase-chain-reaction assay with sequencing, and for clinical symptoms, by means of questionnaires.

RESULTS

A total of 1175 personnel were at risk across the four sites, with 1100 receiving oseltamivir prophylaxis. A total of 75 personnel (6.4%) were infected before the intervention, and 7 (0.6%) after the intervention. There was a significant reduction in the overall reproductive number (the number of new cases attributable to the index case), from 1.91 (95% credible interval, 1.50 to 2.36) before the intervention to 0.11 (95% credible interval, 0.05 to 0.20) after the intervention. Three of the four outbreaks showed a significant reduction in the rate of infection after the intervention. Molecular analysis revealed that all four outbreaks were derived from the New York lineage of the 2009 H1N1 virus and that cases within each outbreak were due to transmission rather than unrelated episodes of infection. Of the 816 personnel treated with oseltamivir who were surveyed, 63 (7.7%) reported mild, nonrespiratory side effects of the drug, with no severe adverse events.

CONCLUSIONS

Oseltamivir ring chemoprophylaxis, together with prompt identification and isolation of infected personnel, was effective in reducing the impact of outbreaks of 2009 H1N1 influenza in semiclosed settings.

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THE 2009 PANDEMIC INFLUENZA A (H1N1) virus has spread rapidly worldwide, despite initial attempts at containment through screening, isolation, and quarantine.[1-3] Many countries moved rapidly into the mitigation phase after the outbreak was detected, which affected essential services, especially in the health and education sectors. Mexico, the first country affected, shut down all major public services for a week to halt transmission of the virus. Other large outbreaks in population centers had a similar effect on essential services. Even though pandemic vaccines are available, the lack of availability during a pandemic results in incomplete global protection.

Mathematical models of the efficacy of containment measures in an influenza epicenter have been described,[4,5] although these measures ultimately proved ineffective at preventing the spread of the 2009 pandemic H1N1 virus. However, containment measures may be effective within specific closed environments, such as schools, health care settings, or military installations, all of which have a high risk of transmission.[6] Chemoprophylaxis with a neuraminidase inhibitor has been effective in preventing the household transmission of influenza,[7] and modeling studies have predicted that well-timed chemoprophylaxis could significantly reduce the rate of absenteeism among health care workers due to illness, to maintain business continuity.[8]

Although antiviral "ring chemoprophylaxis" strategies (aimed at geographically targeted containment by means of prophylaxis) were predicted to be effective in mathematical models, data are needed to document their actual effectiveness during a pandemic. We therefore describe our experience in responding to four outbreaks of the 2009 pandemic influenza A (H1N1) virus in military camps (including one in a health care setting) and evaluate the role of oseltamivir "ring chemoprophylaxis" in attenuating transmission of the virus.

METHODS

Singapore is a city–state of 4.84 million people.[9] All Singaporean men perform 2 years of military service after high school, at 18 to 19 years of age. Most military personnel live in barracks-style accommodations on weekdays and return home on weekends, resulting in an interaction between the military community and the Singapore population.

Singapore identified its first imported case of infection with the 2009 pandemic influenza A (H1N1) virus on May 27, 2009,[10] and the first transmission to the local community was reported on June 18, 2009.[11] In line with World Health Organization (WHO) recommendations,[12] Singapore began the transition to mitigation on July 1, 2009.[13] The Singapore Armed Forces (SAF) identified its first imported case of infection on June 15, 2009, and its first four outbreak clusters (outbreaks I, II, III, and IV) involving local transmission from June 22 to 25, 2009.

NATIONAL PROTOCOLS AND MANAGEMENT

A suspected case of 2009 H1N1 influenza was defined as influenza-like illness (temperature ≥38.0°C with cough or sore throat) with an onset of symptoms within 7 days after travel to an affected area, close contact with a person with confirmed infection, or contact with a local cluster of infected persons.[14] Laboratory confirmation of suspected cases was performed by means of real-time reverse-transcriptase–polymerase-chain-reaction (RT-PCR) assay or viral culture.[14]

Until July 1, 2009, all persons with suspected infection with the 2009 H1N1 virus were screened with the use of RT-PCR assay, according to national protocols,[15] and patients with confirmed infection were isolated in hospitals to prevent transmission. Contact tracing was performed to identify close contacts, defined as persons who had had unprotected exposure, within 2 m, to an infected patient for 1 hour or more since the day before the onset of symptoms.[10] Most contacts were quarantined at home for a 7-day period.

SAF PROTOCOL AND MANAGEMENT

Performing its function as a critical national resource, the SAF implemented additional interventions to contain the spread of the 2009 H1N1 virus. Primarily, "ring prophylaxis" with oseltamivir (Tamiflu, Roche), at a dose of 75 mg daily, was administered to coworkers of the patient with confirmed infection for a period of 10 days after exposure.[16] The oseltamivir had been purchased and stockpiled several years previously as part of the SAF influenza-pandemic preparedness plan. A coworker was defined as a member of the same military unit, where contact opportunities were substantial even if they did not fulfill the Singapore Ministry of Health criteria for close contact. This wider definition was prompted by difficulties in identifying actual contacts and the

practicalities of rapidly administering prophylaxis. Larger prophylaxis rings were instituted if cases were present in multiple units. In addition, interactions between affected units and other units were reduced within the camp, by allocating to each unit different times of arrival, departure, and meal delivery.

EPIDEMIOLOGIC INVESTIGATION

Our investigation of the outbreaks was approved by the SAF Joint Medical Committee, as well as the National University of Singapore and the Australian National University institutional review boards. Written informed consent was obtained from all persons for whom follow-up nasopharyngeal swabs were obtained, and oral assent was provided by all others during the surveys.

The four outbreaks occurred in different locations: one in each of three military units and one at a camp medical center. All personnel with suspected infection were tested and isolated in the hospital if the test was positive. In addition, all asymptomatic personnel in the same unit were screened through the collection of nasopharyngeal swabs, three times a week, to detect subclinical infections.[17] A written questionnaire was administered at each screening visit, as well as after the completion of prophylaxis, to collect data on demographic characteristics, medical history, activity patterns, and clinical symptoms. Screening was performed until no additional cases were identified for 3 days after the last previously identified case or after the end of the 10-day prophylaxis period, whichever was later. After the prophylaxis period, a telephone questionnaire was administered to personnel who had left camp before the screening was completed.

MOLECULAR DIAGNOSIS AND SEQUENCING

Nasopharyngeal swabs were collected, resuspended in 2.0 ml of viral-transport medium, and sent for RT-PCR testing, all within a 24-hour period. The RT-PCR assay involved protocols with the swine H1 forward–reverse primer set and probe.[18] Positive samples with sufficient RNA underwent whole-genome sequencing according to a previously reported approach.[19] The resulting sequences were used to generate phylogenetic trees with the use of Molecular Evolutionary Genetics Analysis 4 software.[20] All sequenced samples were screened for known and suspected mutations that would confer oseltamivir resistance, including the H274Y mutation. Additional methods are described in the Supplementary Appendix (available with the full text of this article at NEJM.org).

STATISTICAL ANALYSIS

Following the statistical argument of Cauchemez and colleagues,[21] we assumed that each case of 2009 H1N1 influenza leads to new cases, distributed as a Poisson variate with a mean of λ or $\lambda\theta$ in the absence or presence of intervention, respectively, as well as a specific form for the generation interval. The λ variable represents the reproductive number (the mean number of new cases attributable to the index case) in the absence of intervention, and $\lambda\theta$ the reproductive number after intervention. Analysis was performed according to the Bayesian paradigm,[22] and with the use of the statistical programming language R.[23] The Supplementary Appendix describes that analysis as well as the methods used to quantify the strength of the intervention effect, obtain credible intervals, and evaluate the hypothesis of a reduction in infection rates after intervention (i.e., $\theta<1$). For measures of statistical significance, we report the posterior hypothesis probabilities as described in the Supplementary Appendix.

RESULTS

A total of 82 confirmed cases of infection with the 2009 pandemic influenza A (H1N1) virus were identified during the four outbreaks (Table 1).

OUTBREAK 1

From June 21 to 22, 2009, four personnel (B, C, E, and F in Fig. 1) tested positive for 2009 H1N1 influenza. Three (B, E, and F) had performed overnight guard duty together on June 18, 2009. Four more (A, G, H, and I) were confirmed to be infected during initial investigations. The remaining 208 coworkers were given oseltamivir prophylaxis; of these, 81 were identified as close contacts and were quarantined at home. During the outbreak, three more personnel tested positive, of whom two (D and J) had not been initially identified as close contacts. Of the remaining 205 personnel, 185 (90.2%) completed the course of prophylaxis. Fourteen personnel reported minor respiratory symptoms; 11 tested negative for 2009 H1N1 influenza and 3 were not tested. The other personnel continued working in the camp, and none tested positive, as assessed by testing three consecutive na-

Table 1. Summary of the Four Outbreaks of 2009 H1N1 Influenza and Efficacy of Oseltamivir Prophylaxis and Other Interventions.*

Variable	Total	Outbreak 1	Outbreak 2	Outbreak 3	Outbreak 4
Total no. of personnel	1175	216	47	219	693
Confirmed cases — no. (%)	82 (7.0)	11 (5.1)	6 (12.8)	2 (0.9)	63 (9.1)
Before intervention — no. (%)	75 (6.4)	8 (3.7)	6 (12.8)	2 (0.9)	59 (8.5)
After intervention — no. (%)	7 (0.6)	3 (1.4)	0	0	4 (0.6)
Posterior hypothesis probability	<0.001	0.11	<0.001	<0.001	<0.001
Symptomatic personnel (excluding confirmed cases)					
Tested and negative — no. (%)	23 (2.0)	11 (5.1)	0	1 (0.5)	11 (1.6)
Not tested — no. (%)	47 (4.0)	3 (1.4)	0	4 (1.8)	40 (5.8)
Mild respiratory symptoms only	40 (3.4)	1 (0.5)	0	4 (1.8)	35 (5.1)
Reported fever with respiratory symptoms	7 (0.6)	2 (0.9)	0	0	5 (0.7)
Completion of oseltamivir prophylaxis — no./total no. (%)†	929/974 (95.4)	185/205 (90.2)	41/41 (100)	186/193 (96.4)	517/535 (96.6)
Confirmed cases and symptomatic personnel who were not tested‡					
Total — no./total no.	115/1161	14/216	6/47	5/218	90/680
Before intervention — no./total no. (%)	85/1161 (7.3)	10/216 (4.6)	6/47 (12.8)	3/218 (1.4)	66/680 (9.7)
After intervention — no./total no. (%)	30/1076 (2.8)	4/206 (1.9)	0	2/215 (0.9)	24/614 (3.9)
Posterior hypothesis probability	<0.001	0.02	<0.001	0.09	<0.001

* The posterior hypothesis probabilities were calculated for the comparison of the incidence of infection before intervention and after intervention, as described in the Supplementary Appendix.

† The number of subjects who completed the oseltamivir prophylaxis regimen excludes those with confirmed infections and those who could not be contacted.

‡ The number of confirmed cases and symptomatic personnel who were not tested excludes 14 symptomatic personnel who could not remember the date of onset of their illness. The percentage of confirmed cases and symptomatic personnel who were not tested before intervention is based on the total number with data; the percentage after intervention is based on the total number with data minus the number identified before intervention.

sopharyngeal swabs obtained over a 1-week period. Overall, 11 of the 216 personnel (5.1%) were infected (Fig. 1).

OUTBREAK 2

In a military medical center, 6 of 47 health care workers tested positive from June 24 to 25, 2009. Because health care workers were essential for the medical center to function, oseltamivir prophylaxis was administered to the remaining 41 personnel, who continued to work while wearing personal protective equipment (N95 mask, gloves, gown, and cap). All 41 health care workers completed the prophylaxis, and none had evidence of infection on testing of three consecutive nasopharyngeal swabs obtained over a 1-week period.

OUTBREAK 3

On June 23, 2009, the index patient presented with influenza-like illness and tested positive. On June 20, 2009, he had visited a nightclub in Singapore (where there was a separate outbreak).[24] One other asymptomatic case in the unit was confirmed during initial investigations. The remaining 217 personnel in the unit were immediately started on prophylaxis, and active surveillance was performed, consisting of testing of two nasopharyngeal swabs obtained over a 3-day period. None tested positive. After prophylaxis, telephone surveillance was performed, with 193 of the 217 personnel (88.9%) successfully contacted; 186 of 193 (96.4%) had completed the prophylaxis. Only one soldier reported fever; he tested negative.

OUTBREAK 4

A unit of 693 army-reserve personnel entered the camp from the community on June 22, 2009, for 5 days of training. From June 25 to 26, a total of 59 personnel presented with fever and respiratory symptoms and tested positive. The index patient

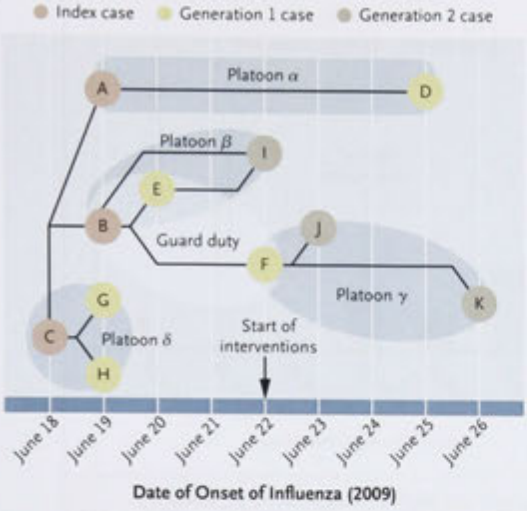

Figure 1. Timing of Events and Cases during Outbreak 1, According to Date of Onset of Influenza.

Generations 1 and 2 are the first and second generations, respectively, of 2009 H1N1 influenza spread from the three presumed index cases.

could not be conclusively identified. Prophylaxis was begun in the remaining 634 personnel, who were given home leave after completion of training on June 26. They were followed by means of telephone surveillance. Throughout the outbreak period, a total of 63 personnel (9.1%) had confirmed infection (Fig. 2). After prophylaxis was completed, the remaining 630 unaffected personnel were surveyed by means of telephone; 535 (84.9%) responded, of whom 517 (96.6%) reported having completed the prophylaxis. A total of 41 respondents reported having respiratory symptoms, and 10 reported having fever with respiratory symptoms. Of these personnel, six and five, respectively, were tested; all tests were negative.

MOLECULAR SEQUENCING

The use of whole-genome sequencing allowed for a molecular epidemiologic analysis, as previously described.[25] Whole-genome sequences were used to identify the relatedness of the isolated viruses and to suggest clusters of transmission to further describe the conditions of the outbreak (Fig. 3).

Each of the four outbreaks formed a distinct cluster, with the closest international strains derived from the New York lineage A/New York/18/2009(H1N1). Outbreak 4 comprised two viral clusters, one New York–like and the other similar to the Singapore local-nightclub cluster[24]; strains isolated during the other outbreaks matched Singapore strains closely. The whole-genome sequences of viruses from outbreak 2 were tightly clustered, suggesting a single causal virus, whereas the local components of outbreaks 1, 3, and 4 were from introductions of highly related Singapore strains, not repeated introductions of distinct viruses. The molecular evidence strongly supports the results of our epidemiologic investigation, which bear out the premise that the outbreaks consisted of transmitted cases of infection rather than unrelated cases.

All seven confirmed cases with an onset after oseltamivir prophylaxis occurred within 4 days after the intervention. The affected patients had complied with the prophylaxis; at the time of infection, they were switched to a treatment dose. In six of the seven cases, there was sufficient genetic material for sequencing. None of the sequenced samples (37 in total, including these 6) had any known or suspected mutations that might have conferred resistance to oseltamivir (including the H274Y mutation).

RATES OF INFECTION AND EFFICACY OF INTERVENTIONS

The overall proportion of personnel with infection before the oseltamivir prophylaxis and the other interventions were instituted was 6.4% across all four military units (Table 1). After prophylaxis was begun, in combination with home leave coordination of schedules to avoid contact among the units at the camp, seven more cases were confirmed (0.6% of the study population). After intervention, the infection rate was reduced to 5.9% of the original rate (95% credible interval, 2.5 to 10.9), (posterior hypothesis probability, <0.001).

Guided by the phylogenetic analyses, we used mathematical modeling to investigate the effect of the interventions on the course of the outbreaks. If we considered only confirmed cases, the global estimate of the reproductive number before intervention was 1.91 (95% credible interval, 1.50 to 2.36). There was a significant reduction in the reproductive number after intervention, to 0.11 (95% credible interval, 0.05 to 0.20) (posterior hypothesis probability, <0.001). If untested, symptomatic cases were included, the reproductive

number before the interventions was 1.85 (95% credible interval, 1.48 to 2.24), with a significant reduction after intervention, to 0.28 (95% credible interval, 0.20 to 0.38) (posterior hypothesis probability, <0.001).

The rate of infection was clearly reduced as a result of interventions in outbreaks 2, 3, and 4 (Table 1). In outbreak 4, ring prophylaxis coincided with the sending home of personnel; thus, to test the effectiveness of prophylaxis, we projected the distribution of one further generation of cases, using the posterior mean of the reproductive number during the preintervention period (Fig. 2). The two distributions we estimated represent what we would expect if the apparent efficacy of the interventions was due to chance alone or due to the isolation measures, not the oseltamivir prophylaxis. The large discrepancy between these distributions and the observed trajectory of the epidemic strongly suggests that the sharp drop in rate of infection was due to prophylaxis, which reduced the transmission of the virus, as well as isolation (rather than isolation alone).

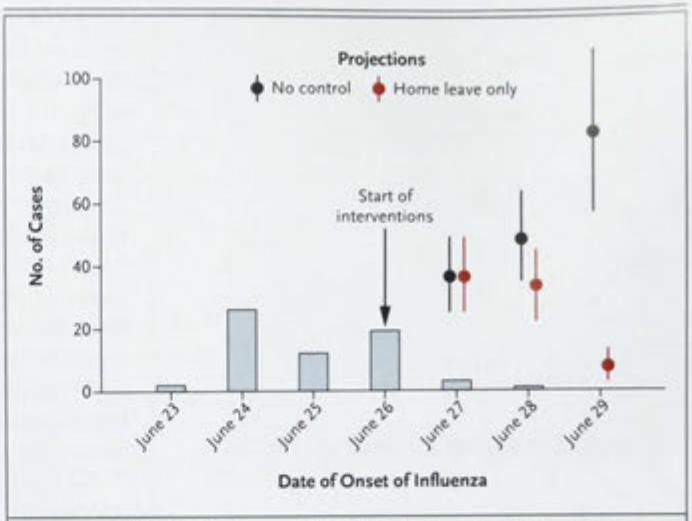

Figure 2. Epidemiologic Data and Model Projections for Outbreak 4, According to Date of Onset of Influenza.

The numbers of cases of 2009 H1N1 influenza during outbreak 4 are shown. Also shown (as circles) are predicted numbers of cases on the basis of assumptions that the apparent effect of the interventions was due to either chance alone ("no control") or to the release of the personnel to home ("home leave only"), rather than to the oseltamivir prophylaxis. I bars indicate 95% credible intervals of the predicted values.

SIDE EFFECTS OF OSELTAMIVIR

We surveyed a total of 816 personnel for side effects of oseltamivir prophylaxis. In all, 63 (7.7%) reported mild, nonrespiratory symptoms (Table 2). No neuropsychiatric events or severe adverse events were reported.

DISCUSSION

Many essential services are provided by persons who work in semiclosed or closed environments where influenza outbreaks can be rapid and severe.[6,26] In an influenza outbreak among Taiwanese military recruits, the rate of infection was 57.7%[27]; an influenza A (H3N2) outbreak on a U.S. Navy ship had an infection rate of 42%.[28] High rates of infection are also reported at schools, which are similarly enclosed. One boarding school had 56 cases (in 6.5% of the population) a week after the index case occurred,[29] and another had an overall rate of infection of 71%.[30] During a New York City school outbreak of the 2009 pandemic influenza A (H1N1) virus, 35% of students reported symptoms of influenza-like illness.[31] In our study, during outbreak 4, 59 cases occurred within 4 days after the first contact with the index patient.

Two modeling studies of the containment of pandemic epicenters, although not specifically based on closed communities, have predicted the effectiveness of ring prophylaxis.[4,5] The effectiveness of antiviral prophylaxis has not been well documented in outbreak situations outside the household setting.[32] The use of postexposure prophylaxis with oseltamivir in close household contacts of patients with seasonal influenza resulted in protective efficacies of 68%[7] and 89%[33] against clinically diagnosed influenza. Early prophylaxis with amantadine also reduced the incidence of influenza, and its associated mortality rate, in outbreaks at long-term care facilities.[34]

For the 2009 influenza pandemic, H1N1 observations suggest that antiviral prophylaxis administered in contacts within households, schools, and workplaces is effective in slowing transmission.[35] In the present study, we have shown that ring prophylaxis with oseltamivir given after exposure in military camps, including a health care setting, was effective, allowing training and operations to continue while substantially reducing the risk of further generations of cases during prophylaxis. The settings studied have the poten-

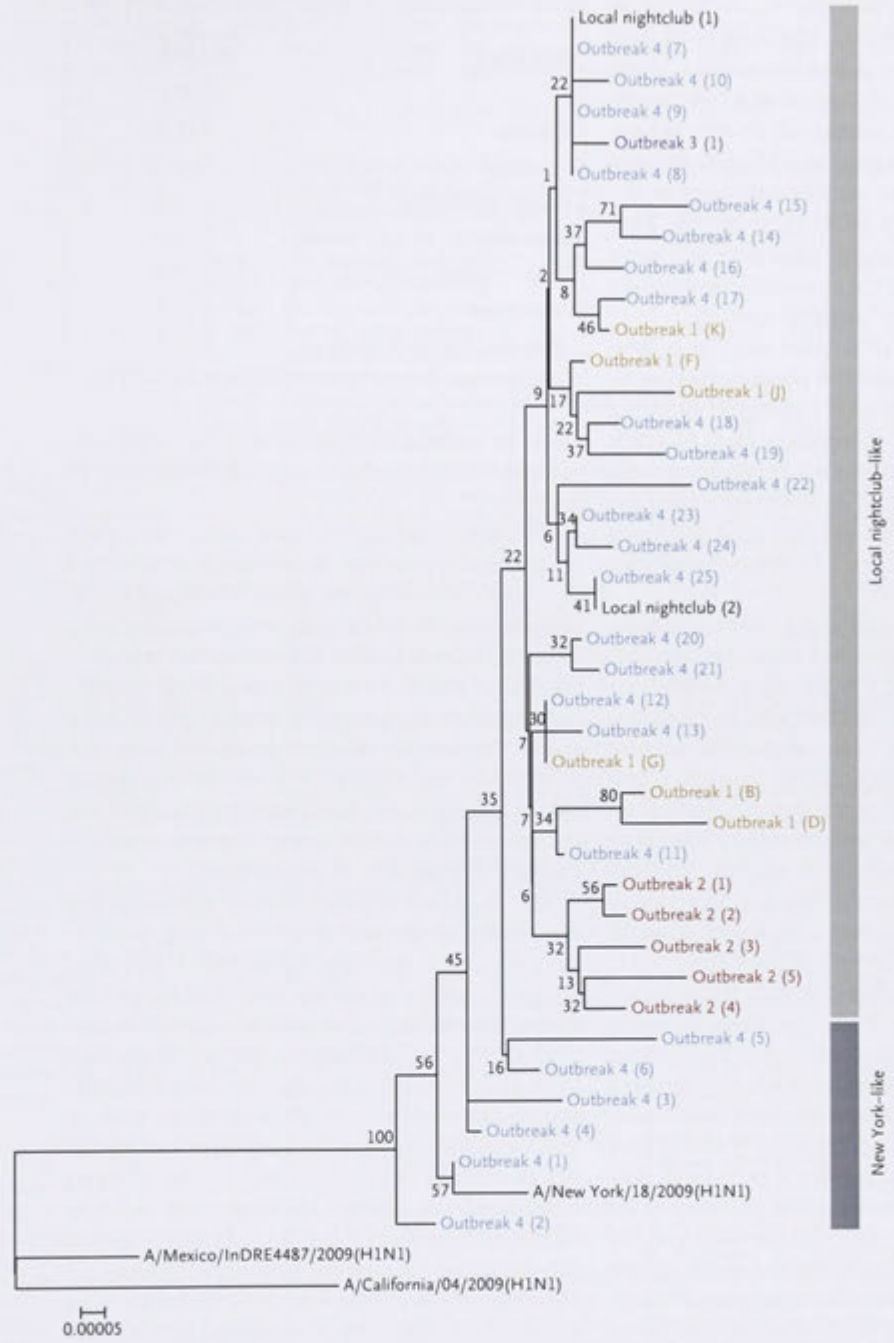

Figure 3. Phylogenetic Relationships among the Viruses Identified during the Four Outbreaks with the Use of Whole-Genome Sequencing.

Numbers in parentheses indicate the identifier of the substrain; the letters refer to the patient identifiers used in Figure 1. Strains from each outbreak are denoted in a unique color. Numbers at each node in the tree indicate the bootstrap value (reflecting the robustness of the evidence supporting the clade of which that node is the root). The scale bar denotes the number of DNA base substitutions (a measure of evolutionary divergence).

tial for intense transmission and are similar to environments such as hospital wards, boarding schools and other schools, and long-term care facilities. The initial response to outbreak 1 also reflects the limitations of quarantining only people considered to be close contacts of an affected patient, since some cases were identified in patients who were contacts, but not close contacts as defined by the Singapore Ministry of Health. Ring prophylaxis, based on spatial proximity, was more effective in controlling the spread of disease than was an exclusive focus on close contacts.

The pandemic (H1N1) 2009 vaccine is now available[36]; however, antiviral prophylaxis may be considered as an additional strategy in reducing the pandemic's effects, especially in areas in which the supply of vaccine is limited. Furthermore, this strategy may be important in future epidemics and pandemics, either before vaccines are available or when there is a poor match between the vaccine and circulating strains.

The threshold for initiating neuraminidase-inhibitor prophylaxis has not been well defined. For outbreaks 1, 2, and 3 in our study, prophylaxis was initiated early and was followed by rapid cessation of the outbreak. This was possible because of rapid detection through health education, surveillance through daily measurement of temperature and monitoring of symptoms, and laboratory testing. Although outbreak 4 was not detected early, postexposure prophylaxis was effective in breaking the chain of transmission and probably helped prevent a higher rate of infection.

Study limitations include the facts that the data were observational and that multiple interventions were applied simultaneously. The relative strength of the nonpharmaceutical interventions as compared with prophylaxis could only be inferred through modeling. However, it would have been difficult to use prophylaxis as the sole control measure, owing to external pressure to do everything possible to halt transmission and the spontaneous social-distancing measures people take. Although the best efforts were made to ensure consistency of the data collection and use of interventions across the four outbreaks, local circumstances influenced the study activities and should be considered part of any investigation of outbreaks. In addition, monitoring data were incomplete for some outbreaks, because personnel completed their training and were given home leave; we subsequently performed telephone surveillance instead to obtain as much information as possible.

Table 2. Side Effects of Oseltamivir Prophylaxis.

Side Effect	Personnel (N = 816)
	no. (%)
Diarrhea	14 (1.7)
Headache	9 (1.1)
Nausea or vomiting	22 (2.7)
Dizziness	5 (0.6)
Epigastric pain	4 (0.5)
Drowsiness	8 (1.0)
Mild allergic reaction (rash)	6 (0.7)

The use of oseltamivir prophylaxis as a containment measure may be limited to semiclosed or closed communities, since transmission in communities in the general population may subsequently lead to further outbreaks. In the boarding school where the use of amantadine prophylaxis significantly reduced the number of influenza cases, the number of cases increased after the prophylaxis was stopped.[29] However, the overall rate of infection was significantly lower than expected, and cases were spread out over time, reducing the peak rate of absenteeism.

Our experience provides evidence that early case detection and the use of antiviral ring prophylaxis effectively truncate the spread of infection during an epidemic, giving empirical support to theoretical mathematical models. Aggressive prophylaxis may be justifiable to provide protection from an influenza strain that causes severe disease or to protect vulnerable populations such as frail or elderly residents of long-term care facilities or persons in closed or semiclosed environments such as schools, prisons, and military camps. Finally, containing the pandemic's spread may postpone the onset of substantial illness and distribute temporally the burden on the health care system until other control measures, such as vaccine, become available.

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SUPPLEMENTARY APPENDIX

Oseltamivir ring prophylaxis for containment of Influenza A (H1N1-2009) outbreaks
Vernon Lee et al

Laboratory Methods

Molecular Diagnosis

Nucleic acid material for each specimen was extracted using the DNA minikit (Qiagen, Inc, Valencia, CA, USA) according to manufacturer's instructions. Five µl of nucleic acid was subjected to PCR testing for H1N1-2009, according to the SWH1 Forward/Reverse primer set and probe (1). This one-step PCR was performed with the Superscript III RT/Platinum Taq mix (Invitrogen Corporation, CA, USA) on the real-time PCR system (Applied Biosystems 7500, USA).

The PCR thermocycling conditions were 50^0C for 30mins, 95^0C for 2min, followed by 45 cycles of PCR amplification of 95^0C for 15sec and 55^0C for 30sec. A florescence growth curve crossing the threshold line within 40 cycles is indicative of a positive result.

Molecular Sequencing

For the whole genome sequencing, viral RNA from the diagnostic swabs or RNA extracted from MDCK cell cultures was reverse-transcribed to cDNA and then amplified by PCR using H1N1-2009 specific primers. PCR products were sequenced using GIS flu-resequencing microarrays manufactured by Roche Nimblegen in an approach described previously (2,3). These sequences were used to generate phylogenetic trees and genetic relatedness using the Neighbour-Joining algorithm and Maximum Composite Likelihood Nucleotide Substitution model with 10,000 bootstrap replicates using the MEGA 4.0 software (4). More information on the genetic analysis tool can be found at http://mendel.bii.a-star.edu.sg/METHODS/flumapIntro.html.

Statistical Methods

Following the argument in Cauchemez et al (5), we assume that in the absence of control each case creates a number of new cases distributed as a Poisson variate with mean λ, and that the time between onset of the primary and all secondary cases are independently distributed with a discretised gamma distribution, which we parametrised from the posterior mean mean [sic] and variance of the gamma distribution fitted to the data provided by Moser (6). We further assume that after the intervention at time τ (which varies by outbreak), the mean number of cases is $\lambda\theta$. By Rényi's splitting theorem (7), the number of new cases with onset on day j is Poisson with mean

$$\sum_{i \le j} c_i \lambda \theta^{1\{j>\tau\}} w(i-j)$$

where c_t is the number of cases with onset on day t, $w(t)$ is the probability mass function for the generation interval of length t, and $1\{A\} = 1$ if A is true and 0 otherwise. From this the likelihood function follows by taking the product of this over days and outbreaks. The posterior distribution of the parameters conditional on the data is taken to be proportional to this, i.e. a pseudo-objective improper flat prior on the parameters λ and $\lambda\theta$ is assumed. Therefore this analysis was performed within

the Bayesian paradigm using improper flat priors on the parameter space – ie. $p(\lambda,\theta) \propto 1$ if $\lambda>0$ and $\theta>0$, and 0 otherwise; the likelihood function has a finite integral and so the posterior is proper (8).

The posterior distribution is estimated via Markov chain Monte Carlo integration (9). The hypothesis of an effect, $\theta < 1$, is assessed via posterior hypothesis probabilities (10) by direct calculation from the posterior sample of $p(\theta > 1 \mid \text{data})$. In the same fashion, marginal 95% credible intervals are obtained.

To distinguish the effects of ring prophylaxis from that of sending soldiers home for outbreak IV, we calculate the probability distribution of the fitted model from the start of interventions in which only one subsequent generation of contacts is allowed. These use the posterior mean for the secondary infection rate, λ, in the absence of control, and are derived via Monte Carlo simulation.

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Chapter Ten

Seroconversion and Asymptomatic Infections during Oseltamivir Prophylaxis

The landmark study in the previous chapter showed that influenza anti-viral ring prophylaxis within a group can reduce the spread of influenza such that the infection rate during the prophylaxis period is greatly reduced. At the same time, one of the possible issues with anti-viral prophylaxis is the possibility of creating an immunologically naïve group which may result in large outbreaks after ceasing prophylaxis, especially when the group is constantly being exposed to the epidemic ranging in the external population.

A mathematical model that I built several years ago with a colleague, using a representation of a public hospital in Singapore, showed that premature cessation of prophylaxis before the pandemic's peak resulted in higher peak infection rates compared to no prophylaxis use (1). In addition, it may also stretch the overall burden of disease across time, thus reducing the strain on resources and disruption of services at any one point in time (1). However, there is currently a lack of epidemiological evidence to show the infection rates in such groups post-ring prophylaxis, and whether cessation of prophylaxis may cause a surge in cases.

At the same time, prophylaxis failures (failure of the anti-viral prophylaxis to prevent infection while the individual is on prophylaxis) have been documented but most are identified through the development of clinical illness among individuals receiving prophylaxis. However, asymptomatic seroconversion may occur during prophylaxis, either as a result of natural asymptomatic infection which has been known to occur as mentioned in Chapter One, or due to the suppression of clinical symptoms due to the anti-viral drug given as prophylaxis. Such asymptomatic infections may confer immunity against the influenza strain, and increase the overall effectiveness of

antiviral prophylaxis in protecting the group even after cessation. There is likewise a lack of evidence on the seroconversion rates post-prophylaxis and it is important to explore this possibility during a real-life outbreak.

The outbreaks in the Singapore military provide a good opportunity to explore these phenomena as there were many groups that were given anti-viral ring prophylaxis as part of the pandemic response plans for outbreaks in essential personnel during the early stage of the national epidemic. The ring prophylaxis was given for 10 days and since they were started early during the national epidemic, were ceased long before the epidemic's peak and during increasing transmission in the community. The fact that the military servicemen left the camps on weekends meant that they were exposed to the growing epidemic in the general population post-prophylaxis. This presents an ideal opportunity to observe the two issues that were mentioned above. Serial serological samples were obtained from three of these groups and the results of the study are presented in the following manuscript.

The results suggest that prophylaxis does result in asymptomatic infections of about 50%, although the numbers were small. There was also no significant difference in the post-seroconversion geometric mean titers (GMT) of the index cases (with clinical infection) and post-prophylaxis seroconverters compared to those who seroconverted during prophylaxis. This shows that seroconversion during prophylaxis may confer similar protection against future infection. More importantly, there was no substantial increase in the subsequent infection rates post-prophylaxis, even when compared to other cohorts in the military which has been shown in Chapter Five. This provides initial evidence that post-exposure ring prophylaxis, even if for short durations to

terminate the spread of influenza in the early phases of the pandemic, is feasible and does not necessarily result in subsequent spread of the virus. This provides an additional strategy to supplement pre-exposure prophylaxis which has been included in many national preparedness plans but is fraught with issues of timing and availability of sufficient anti-viral drugs.

Study 8

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RESEARCH ARTICLE Open Access

Seroconversion and asymptomatic infections during oseltamivir prophylaxis against Influenza A H1N1 2009

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Abstract

Background: Anti-viral prophylaxis is used to prevent the transmission of influenza. We studied serological confirmation of 2009 Influenza A (H1N1) infections during oseltamivir prophylaxis and after cessation of prophylaxis.

Methods: Between 22 Jun and 16 Jul 09, we performed a cohort study in 3 outbreaks in the Singapore military where post-exposure oseltamivir ring chemoprophylaxis (75 mg daily for 10 days) was administered. The entire cohort was screened by RT-PCR (with HA gene primers) using nasopharyngeal swabs three times a week. Three blood samples were taken for haemagglutination inhibition testing - at the start of outbreak, 2 weeks after completion of 10 day oseltamivir prophylaxis, and 3 weeks after the pandemic's peak in Singapore. Questionnaires were also administered to collect clinical symptoms.

Results: 237 personnel were included for analysis. The overall infection rate of 2009 Influenza A (H1N1) during the three outbreaks was 11.4% (27/237). This included 11 index cases and 16 personnel (7.1%) who developed four-fold or higher rise in antibody titres during oseltamivir prophylaxis. Of these 16 personnel, 8 (3.5%) were symptomatic while the remaining 8 personnel (3.5%) were asymptomatic and tested negative on PCR. Post-cessation of prophylaxis, an additional 23 (12.1%) seroconverted. There was no significant difference in mean fold-rise in GMT between those who seroconverted during and post-prophylaxis (11.3 vs 11.7, $p = 0.888$). No allergic, neuropsychiatric or other severe side-effects were noted.

Conclusions: Post-exposure oseltamivir prophylaxis reduced the rate of infection during outbreaks, and did not substantially increase subsequent infection rates upon cessation. Asymptomatic infections occur during prophylaxis, which may confer protection against future infection. Post-exposure prophylaxis is effective as a measure in mitigating pandemic influenza outbreaks.

Background

Anti-viral prophylaxis has been used as a strategy to prevent the transmission and spread of influenza. Post-exposure prophylaxis with oseltamivir, a commonly used neuraminidase-inhibitor, has been shown to be effective in preventing the development of clinical disease against seasonal influenza when used against household contacts [1,2]. Pre-exposure prophylaxis has also been successfully used in the community [3], and in households [4] to prevent transmission of influenza. For the 2009 pandemic, post-exposure prophylaxis has been used in household and community contacts of pandemic influenza cases [5], as well as in pandemic influenza outbreaks in closed environments [6].

One of the uncertainties with prophylaxis is the risk of maintaining an immunologically naïve population which may increase the possibility of outbreaks after the cessation of prophylaxis. One mathematical model showed that premature cessation of prophylaxis before the pandemic's peak resulted in higher peak infection rates compared to no prophylaxis use [7]. However, prophylaxis may delay the spread of the virus such that the overall

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infection rate in the affected group is reduced, and may spread out the burden of disease, thus reducing the strain on resources and disruption of services. Currently, there is little evidence on the actual outcome of prophylaxis in such situations.

Chemoprophylaxis failures have been previously documented but mostly by the development of clinical influenza illness among individuals receiving prophylaxis [1,4]. However, influenza may also result in asymptomatic infections [8], and one previous study showed that asymptomatic infections while receiving oseltamivir prophylaxis do occur [3]. Asymptomatic sero-conversion may confer protection and increase the overall effectiveness of antiviral prophylaxis in protecting individuals and cohorts even after cessation by increasing herd immunity.

We performed a study in the tropical city-state of Singapore to determine symptomatic and asymptomatic serological confirmation of 2009 Influenza A (H1N1) infections during oseltamivir prophylaxis and after cessation of prophylaxis, in 3 separate outbreaks. The findings will be important in the application of future chemoprophylaxis strategies.

Methods

We performed an observational cohort study in the Singapore military from 22 Jun 09 to 16 Jul 09. The Singapore military has a mix of regular employees and conscript personnel where all males are required to serve after high school. These personnel live in camps during the week and return home on weekends, resulting in a semi-closed community with exposures to the national community. The Singapore military identified its first imported case of 2009 Influenza A (H1N1) on 15 Jun 2009, and on 22 Jun 2009 identified its first outbreak cluster with local transmission.

In line with national protocols, cases of 2009 Influenza A (H1N1) were determined via laboratory confirmed infection by real-time reverse transcription polymerase chain reaction (RT-PCR) or viral culture [9]. In addition to the national protocol of hospital or home isolation of cases during the early containment phase of the local epidemic [10], the Singapore military used the strategy of geographical oseltamivir ring chemoprophylaxis of affected military units with 10 days of oseltamivir 75 mg once a day, and cohorting of the entire units (as a form of social distancing) to prevent spread.

Epidemiological Investigations

The study was performed among 252 personnel involved in 3 separate 2009 Influenza A (H1N1) outbreaks, whereby post-exposure oseltamivir ring chemoprophylaxis was administered. At the onset of each outbreak, a 10 day course of post-exposure oseltamivir chemoprophylaxis was given to each cohort and they continued to function in their normal capacity. The entire cohort was screened by RT-PCR using nasopharyngeal swabs three times a week, until no further positive cases were discovered for three days. All confirmed cases were given a minimum of 7 days home medical leave. The rest of the cohort continued their regular schedule, including staying in camp during weekdays and returning home during weekends.

In addition, three samples of 5 to 10 ml of venous blood were taken from each participant in for serological testing. The first baseline sample was taken at the start of outbreak. The second sample was taken between 2 to 3 weeks after the completion of oseltamivir prophylaxis. This timeframe allowed sufficient time for seroconversion from infections during prophylaxis, while reducing the likelihood of seroconversion from infections after prophylaxis [11]. The third sample was taken 3 weeks after the peak of the pandemic in Singapore [12], between 4 to 6 weeks after the completion of prophylaxis, to assess for any additional seroconversion after prophyalxis. Questionnaires were administered to collect data on demographics, medical history, and clinical symptoms.

Written informed consent was obtained from participants, and the study was approved by the Singapore military's Joint Medical Committee (Research) and the Australian National University's ethics review board.

Laboratory Analysis

The nasopharyngeal swabs collected were resuspended in 3.0 ml of universal transport medium (Copan Diagnostics Inc., USA) and sent for laboratory testing. Total nucleic acid material was extracted using the DNA minikit (Qiagen, Inc, Valencia, CA, USA) according to manufacturer's instructions and subjected to real-time PCR testing for the presence of H1N1-2009 [13]. Briefly, 5ul of nucleic extract was PCR-amplified with 0.8 uM of each of the forward (5'-GAC AAA ATA ACA AAC GAA GCA ACT GG - 3') and reverse primers (5'-GGG AGG CTG TTT ATA GCA CC-3') in the presence of 0.2 uM probe (5'-6-carboxyfluorescein-GCA TTC GCA AT(BHQ)G GAA AGA AAT GCT GG -3') using the Superscript III RT/Platinum Taq mix (Invitrogen Corporation, CA, USA) according to manufacturer's instructions. The reverse transcription (RT) was carried out at 50°C for 30 mins, the reaction denatured at 95°C for 2 mins, and PCR-amplified with 50 cycles consisting of 95°C for 15 sec and 55°C for 30 sec. The RT-PCR testing was carried out on a real-time PCR system (Applied Biosystems 7500, USA), A positive result is defined by a fluorescence growth curve that crosses the threshold line within 40 cycles. Sensitivity of this assay is about 100 copies of RNA genome equivalents per reaction (95% confidence level) [14].

For the blood samples, serum was extracted and tested by haemagglutination inhibition (HI) according to stan-

dard protocols (WHO CC, 1982) at the WHO Collaborating Center for Reference and Research for Influenza in Melbourne, Australia. The serum was pretreated with receptor destroying enzyme (RDE) (Deka Seiken Co. Ltd., Tokyo, Japan) at 1:4 (volume/volume), and the enzyme inactivated by addition of an equal volume of 1.6% trisodium citrate (Ajax Chemicals, Australia). Egg-grown A/California/7/2009 A(H1N1-2009) virus was purified by sucrose gradient, concentrated and inactivated with β-propiolactone, to create an influenza zonal pool preparation (a gift from CSL, Australia). 25 μL of Influenza Zonal Pool-A/California/7/2009 virus was incubated with an equal volume of RDE-treated serum, titrated in two-fold dilutions in phosphate buffer solution from 1:10 to 1:1280, and incubated for 1 hour. 25 μl 1% (v/v) turkey red blood cells was added to each well and read after 30 minutes. Controls for the HI assay were performed with positive ferret sera (sera collected from naive ferrets infected with A/California/7/2009 H1N1 pandemic virus and bled 14 days later), positive human sera from RT-PCR positive individuals collected in the convalescent phase, and negative human sera collected from non-infected individuals. Positive sera had high titres by both HI and MN assays against pandemic H1N1 viruses. Titres were expressed as the reciprocal of the highest dilution of serum where haemagglutination was prevented. Individual seroconversion was indicated by a four-fold or greater rise in titres between successive samples.

Statistical Analysis

The data was analyzed using the Statistical Package for the Social Sciences (SPSS, version 16.0, Chicago, IL) with the level of significance set at 0.05. Categorical variables were summarized as percentages and continuous variables as means with standard error (SE); the Student's T-test was used to investigate the relationship between continuous variables.

Results

Outbreaks A, B and C occurred in 3 separate units on 22 Jun 09, 9 Jul 09 and 16 Jul 09 respectively. Prior to these outbreaks, there were no increases of influenza-like illness or respiratory illness cases in these units, nor were there any confirmed cases of 2009 Influenza A (H1N1). Of the 252 personnel initially identified and sampled; 15 personnel were subsequently excluded as they had completed their conscript service and left the military before completion of the study (Figure 1). The final study population consisted of 237 personnel of which 11 personnel were index cases of the outbreaks. These index cases were started on treatment dose of oseltamivir (75 mg twice daily for 5 days) and given medical leave at the onset of the outbreak and thus did not have any baseline serology taken.

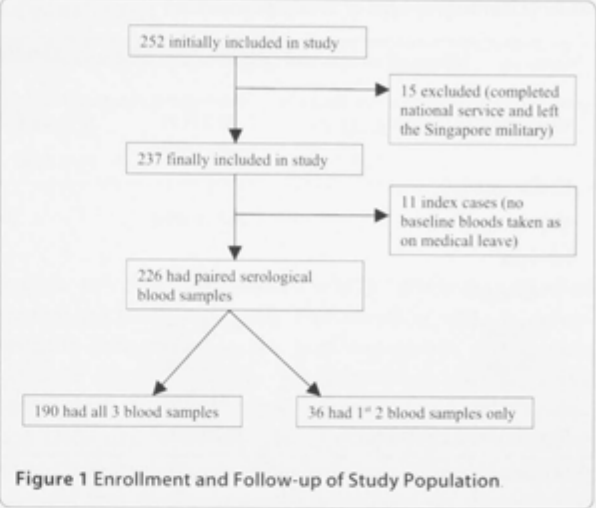

Figure 1 Enrollment and Follow-up of Study Population

The mean age of the study population was 21.2 years old (range 18.7-30.8) (Table 1) and all were male, reflecting the composition of the military. The ethnic make-up was similar to the general Singapore population. Twenty-three personnel (9.7%) had a history of asthma and 1 (0.4%) each had hypertension and IgA nephropathy; no other relevant medical conditions were present.

Seroconversion During Prophylaxis (Table 2)

The overall infection rate of 2009 Influenza A (H1N1) during the three outbreaks was 11.4% (27 personnel, including 11 index cases). A total of 16 personnel (excluding index cases) developed a four-fold or higher rise in antibody titres between the first and second blood sample, indicating infection whilst on oseltamivir prophylaxis. Of these, 8 (3.5% of the population) were symptomatic - 6 had fever together with respiratory symptoms while 2 had only respiratory symptoms. The remaining 8 personnel (3.5%) were asymptomatic and tested negative on PCR from consecutive nasopharyngeal swabs.

Seroconversion Post-Prophyalxis

An additional 23 (12.1%) patients developed 4-fold rise in antibody titres between the second and third blood sample, indicating infection after the cessation of prophylaxis and up to the peak of the epidemic wave. Four (2.1%) were symptomatic.

Antibody Titres

The baseline and post-seroconversion geometric mean titres (GMT) of those who seroconverted during prophylaxis was 7.4 and 59.1 respectively (Table 3). The baseline and post-seroconversion GMT of those who seroconverted post-prophylaxis was 6.6 and 62.9 respectively. There was no significant difference in mean fold-rise in GMT between the two groups (11.3 vs 11.7, $p = 0.888$).

Table 1: Demographics of study population

	Overall (n = 237)	*Outbreak A (n = 149)*	*Outbreak B (n = 42)*	*Outbreak C (n = 46)*
Mean age (SE) (range)	21.2 (1.7) (18.7-30.8)	21.1 (2.1) (18.7-30.8)	21.2 (0.8) (20.2-23.8)	21.2 (0.5) (20.1-22.4)
Median age (yr)				
Male	237 (100%)	149 (100%)	42 (100%)	46 (100%)
Ethnicity				
Chinese	175 (73.8%)	100 (67.1%)	36 (85.7%)	39 (84.8%)
Malay	41 (17.3%)	34 (22.8%)	4 (9.5%)	3 (6.5%)
Indian	12 (5.1%)	8 (5.4%)	1 (2.4%)	3 (6.5%)
Others	9 (3.8%)	7 (4.7%)	1 (2.4%)	1 (2.2%)
Significant medical history	25 (10.5%)*	21 (14.1%)	4 (9.5%)	0 (0.0%)

*All cases of significant medical history were asthma except 1 case of hypertension and 1 case of IgA nephropathy

Ten index cases (ie given treatment dose of oseltamivir) had a single post-seroconversion blood sample taken. The post-seroconversion GMT for these index cases was 65.0 (SE 6.8) compared to 59.1 (SE 6.1) in those who seroconverted during prophylaxis (p = 0.590).

Compliance and Side Effects

Of the 237 who started prophylaxis, 228 personnel (96.2%) completed the full course of oseltamivir. Nine personnel (3.8%) did not complete the full course due to non-compliance and side effects; 5 (2.1%) complained of nausea/vomiting. Of these 9 personnel, one was among the symptomatic individuals who seroconverted, while the other 8 did not have any symptoms and did not seroconvert. No allergic, neuropsychiatric or other severe side-effects were noted.

Discussion

Prophylaxis with oseltamivir has been shown to be effective in reducing the immediate spread of influenza in community and household settings during the period of administration [3,4]. However, the effectiveness of oseltamivir in reducing subsequent infection rates has not been widely studied. Our study showed that prophylaxis may be effective not only in reducing the spread of influenza during a localized outbreak, but also after cessation of prophylaxis during the overall epidemic. In our study, the

Table 2: Seroconversion during and post-prophylaxis in the study population

	Overall (n = 237)	*Outbreak A (n = 149)*	*Outbreak B (n = 42)*	*Outbreak C (n = 46)*
Date of 1st blood sample	23 Jun-16 Jul 10	23 Jun 10	9 Jul 10	16 Jul 10
Date of 2nd blood sample	13 Jul-6 Aug 10	13 Jul 10	30 Jul 10	6 Aug 10
Date of 3rd blood sample	21-25 Aug 10	21 Aug 10	25 Aug 10	25 Aug 10
Seroconversion during prophylaxis (second vs first samples)*				
Total	16/226 (7.1%)	10/141 (7.1%)	4/40 (10%)	2/45 (4.4%)
Symptomatic	8/226 (3.5%)	3/141 (2.1%)	3/40 (7.5%)	2/45 (4.4%)
Asymptomatic (and RT-PCR negative)	8/226 (3.5%)	7/141 (5.0%)	1/40 (2.5%)	0/45 (0.0%)
Overall infection rate during outbreak (serological and index cases)	27/237 (11.4%)	18/149 (12.1%)	6/42 (14.3%)	3/46 (6.5%)
Seroconversion post-prophylaxis (third vs second samples)				
Total	23/190 (12.1%)	16/115 (13.9%)	1/34 (2.9%)	6/41 (14.6%)
Symptomatic	4/190 (2.1%)	2/115 (1.7%)	1/34 (2.9%)	1/41 (2.4%)
Asymptomatic	19/190 (10.0%)	14/115 (12.2%)	0/34 (0.0%)	5/41 (12.2%)

*Excluding index cases

Table 3: Comparison of change in antibody titres during and post-prophylaxis

	Baseline GMT(SE)	Post-seroconversion GMT (SE)	Mean fold-rise in titres (SE)	p- value*
Seroconversion during prophylaxis	7.4 (5.8)	59.1 (6.1)	11.3 (2.7)	0.888
Seroconversion post-prophylaxis	6.6 (5.7)	62.9 (5.7)	11.7 (1.5)	

*Comparing mean fold-rise in titres

overall infection rate during the outbreak was 11.4%. This was lower than clinical attack rates in other seasonal influenza outbreaks documented in the military- 57.7% among Taiwanese military recruits [15] and 42% on a navy ship [16]. Our infection rates were also lower when compared to similar 2009 Influenza A (H1N1) outbreaks in other closed communities - >30% attack rate in a school outbreak [17] and 13-17% among household contacts (which consisted of older age groups) [18].

The seroconversion rate after the cessation of prophylaxis until after the community epidemic's peak was 12.1%, which was similar to that of the initial outbreak (11.4%). In addition, the overall combined infection rate throughout the entire epidemic of 21.1% (50/237) was lower than that of other similar military cohorts surveyed in the Singapore military during the same period with a seroconversion rate of 28% [19]. The latter cohorts did not receive early oseltamivir prophylaxis. This shows that anti-viral prophylaxis did not render the population more susceptible to further outbreaks even though prophylaxis was stopped 1-4 weeks before the peak of the epidemic. On the contrary, anti-viral prophylaxis allowed cases to be spread out across time, reducing peak absenteeism and disruptions to the military or business continuity. A previous study in another closed environment, a boarding school, using amantadine post-exposure prophylaxis for seasonal influenza A/H3N2 modeled that prophylaxis reduced the number of clinical influenza-like illness cases during its use by approximately 83.7%, and although the number of cases increased upon cessation of prophylaxis, the overall clinical attack rates were 21.7%, which was lower than predicted using previous outbreaks for comparison [20].

Asymptomatic, RT-PCR negative seroconversion occurred in 3.5% of the participants during oseltamivir prophylaxis. This shows likely exposure to and infection with 2009 Influenza A (H1N1), and the subsequent development of antibodies which may be protective, without increasing transmission. Furthermore, we found that the antibody titres in those who seroconverted during prophylaxis were not significantly different from those who seroconverted after cessation of prophylaxis. As such, in addition to preventing clinical infection, prophylaxis may also result in asymptomatic infection and subsequent immunity which provides individual protection against further infection after cessation of prophylaxis, as well as increasing herd immunity. The identical rates of symptomatic and asymptomatic seroconversions during prophylaxis show that the proportion of asymptomatic infection is substantial and must be considered during any influenza outbreak [21]. Our findings are similar to the another study by Hayden and colleagues, which showed that in the general community during seasonal epidemic influenza, 2.3% to 2.5% of those who received oseltamivir prophylaxis had asymptomatic infection, although this was not significant compared to those on placebo [3].

Our study provides evidence on serological infections and asymptomatic seroconversion while on oseltamivir prophylaxis, incorporating serological, PCR and clinical data. However, there are some limitations of this study. The lack of planned control groups which makes it difficult to assess the likely exposures and infection rates within similar settings, but previous experiences have suggested that exposures and infection rates during the initial outbreak phase are high in closed environments [15-17]. In addition, the age group in these outbreaks is limited to young adults. Additional studies should be performed in different populations and age groups, with comparison groups to determine the overall effectiveness of prophylaxis in reducing clinical infections and promoting immunity.

Conclusion

Our study showed that post-exposure oseltmaivir prophylaxis reduced the rate of infection in a vulnerable population and did not adversely increase subsequent infection rates upon cessation of prophylaxis before the epidemic's peak. In addition, we have shown that asymptomatic seroconversions occur during prophylaxis, which may confer protection against future infection. Post-exposure prophylaxis remains a strategy to consider in preventing the spread of influenza in closed environments and essential personnel populations.

Competing interests

VJL has received research support from GSK. PAT has received research support and honoraria from Baxter, Adamas, Merlion Pharma, and Novartis as well as travel support from Pfizer and Wyeth and sits on the boards of the Asia Pacific Advisory Committee on Influenza and the Asian Hygiene Council. The rest of the authors declare that we do not have any conflict of interests, financial or otherwise, in this study.

Authors' contributions

VJL, JJY conceived the study, collected the data, performed the analysis, and wrote the first draft of the manuscript together. JKT, MIC conceived the study, performed the analysis, and participated in the manuscript writing. GQ, HJH collected the data and performed the analysis. IB, AK, PAK, BHT, PAT performed the analysis and participated in the manuscript writing. All authors have read and approved the final manuscript. The corresponding author had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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Chapter Eleven

Oseltamivir Prophylaxis Failures

While the previous chapters show the effectiveness of oseltamivir ring prophylaxis during influenza outbreaks, there is concern that prophylaxis failures may be due to resistance and these strains will be evolutionarily selected for replication and transmission. There are few documented reports of prophylaxis failures during the 2009 pandemic that explores the possible reasons behind these failures.

The following manuscript presents the laboratory confirmed prophylaxis failures that were identified from groups of military servicemen where ring prophylaxis had been used. Although there were only 10 cases available, these provided sufficient viral material for testing for mutations that confer oseltamivir resistance. As the H274Y mutation was involved in most of the resistant viruses, this was one of the main focuses of the testing. In addition, the whole-of-genome sequencing allowed for the identification of other possible mutations and the effect these may have had on resistance to anti-viral drugs.

The study showed that these failures were primary failures and were unlikely to have originated from viral genetic mutations. Although the sample size is small, it provides some evidence that not all prophylaxis failures are due to mutations, and that primary prophylaxis failures remain the most likely cause for the majority of cases. At the same time, it does not mean that anti-viral prophylaxis should be used indiscriminately due to the still existing possibility of promoting resistance by natural selection, and other side effects. Prophylaxis should instead be considered in situations where the benefits outweigh potential costs, such as to maintain essential services or reduce complications in vulnerable populations. The rational use of anti-

viral medications will ensure that they will continue to be viable as a strategy in the future.

Study 9

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Investigation of causes of oseltamivir chemoprophylaxis failures during influenza A (H1N1-2009) outbreaks

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ABSTRACT

Background: Antiviral post-exposure prophylaxis with oseltamivir has been used as a strategy in mitigating the Influenza A (H1N1-2009) pandemic. There have been few reports of well-documented prophylaxis failures and the reasons for failure.
Objectives: We report herein a series of 10 cases of prophylaxis failures and explore the reasons behind their prophylaxis failure.
Study design: In the early pandemic phase, the military employed oseltamivir post-exposure ring-prophylaxis of affected units. From June 22 to July 30, 2009, cases of laboratory-confirmed prophylaxis failures were identified. Nasopharyngeal swabs were collected and tested by PCR. Samples with sufficient RNA material were sent for whole genome sequencing, and screened for mutations that confer oseltamivir resistance, especially the H275Y mutation.
Results: Ten cases of laboratory-confirmed prophylaxis failure were identified, with a mean age of 22.3 years. One case was asymptomatic; the remaining 9 had fever or cough but without severe complications. The mean duration of exposure before starting oseltamivir was 1.9 days (SD 0.9), while the mean duration of oseltamivir consumption before symptom onset was 1.9 days (SD 1.4). None of the samples had the H275Y mutation or other known mutations that confer resistance. From the whole genome sequencing, several mutations at the HA (T220S, E275V, T333A, D239G); PB2 (K660R, L607V, V292I); NS1 (F103S), and NP (W104G) gene segments were detected, but none of them were likely to result in anti-viral resistance.
Conclusions: Primary prophylaxis failures exhibited mild symptoms without complications; all did *not have the H275Y mutation* and were unlikely to result from other mutations.

1. Background

The Influenza A (H1N1-2009) pandemic resulted in the activation of response measures worldwide,[1,2] with oseltamivir antiviral prophylaxis used frequently. Oseltamivir protects against seasonal influenza when used as post-exposure prophylaxis[3,4] or pre-exposure prophylaxis.[5,6] However, resistance to oseltamivir has been developing, especially in seasonal H1N1 viruses, largely due to the H275Y mutation.[7,8]

Abbreviations: PCR, polymerase chain reaction; DNA, deoxyribonucleic acid; RNA, ribonucleic acid; PDB, Protein Data Bank; BMI, body mass index; SD, standard deviation; NP, nucleoprotein; NS1, non-structural protein 1; WT, wild-type; MT, mutant-type; AA, amino acids.

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Influenza A (H1N1-2009) is resistant to adamantanes but remains largely susceptible to neuraminidase inhibitors[9]; with only sporadic cases of oseltamivir resistance reported worldwide.[10,11] The Singapore military utilized post-exposure oseltamivir prophylaxis as part of its pandemic response plan to prevent the uncontrolled spread of influenza among essential personnel, and some prophylaxis failures occurred. Apart from antiviral resistance,[11] prophylaxis failures may also occur without resistance.[3,4] However, there have been few well-documented reports of prophylaxis failures during the 2009 pandemic.

2. Objectives

We aim to explore possible reasons behind a series of oseltamivir prophylaxis failures among military personnel.

3. Study design

The Singapore military is a conscript service for males after high school. During the pandemic, a confirmed influenza A (H1N1-2009) case was defined as laboratory-confirmed infection by reverse transcriptase-polymerase chain reaction (PCR) or viral culture.[12] In the early pandemic containment phase the military employed the strategy of post-exposure oseltamivir chemoprophylaxis for close contacts of cases, with 10 days of oseltamivir (75 mg daily).[13] Any servicemen on prophylaxis with febrile respiratory illness (reported fever with cough or sore throat) underwent PCR testing. A case of prophylaxis failure was defined as confirmed influenza A (H1N1-2009) with onset of symptoms ≥24 h after commencement of oseltamivir. Prophylaxis failures were converted to treatment doses of oseltamivir (75 mg twice a day for 5 days) upon diagnosis.

From June 22 to July 30, 2009, laboratory-confirmed prophylaxis failure cases were identified and information collected via questionnaire, telephone interview and medical records review; including time from exposure (first contact with the case), risk factors, clinical symptoms, medication compliance, and side-effects. This study was approved by the military's Joint Medical Committee, and the National University of Singapore and Australian National University's ethics board.

3.1. Laboratory analysis and sequencing

Nasopharyngeal swabs, collected from all febrile respiratory illness cases, were tested by real time PCR, using the SWH1 Forward/Reverse primer set and probe, as previously described by the World Health Organization.[14]

Positive samples with sufficient RNA material were subjected to whole genome sequencing to determine the molecular basis for failed prophylaxis, as the causative mutation may be beyond the neuraminidase protein. Viral RNA from diagnostic swabs or RNA extracted from Madin-Darby canine kidney cell cultures was reverse-transcribed to cDNA and then amplified by PCR using influenza A (H1N1-2009) specific primers. The PCR products were sequenced using Genome Institute of Singapore influenza-resequencing microarrays manufactured by Roche Nimblegen, as described previously.[15] This was utilized to screen the positive samples for mutations that conferred oseltamivir resistance, including the H275Y mutation detected in sporadic cases worldwide.[10,11] The possible molecular effect of each identified mutations was then interpreted in the prophylaxis failure context.

3.2. Bioinformatics methods

Structural models were created for the reference strain A/New York/20/2009(H1N1) following a previously described procedure,[16] and where possible, additional 3D structural models were generated to strengthen data interpretation of the molecular effect of the mutations identified. We used BLASTP[17] against the Protein Data Bank (PDB)[18] to identify the most closely related template of known structure and then aligned and modelled the query sequence using MODELLER.[19] Complexes with ligands or host proteins were additionally minimized with short simulated annealing molecular dynamics simulations with the AMBER03 force field as implemented in YASARA[20] that was also used for visualization of structures and mutations.

Table 1
Demographics, clinical characteristics and complications of cases ($n = 10$).

	$n = 10$
Demographics	
Age in years: mean (range) (SD)	22.3 (19.6–30.0) (3.1)
Male	10 (100%)
BMI: mean (range) (SD)	24.3 (15.6–34.3) (6.0)
Smoker	5 (50%)
Comorbidities[a]	1 (10%)
Clinical characteristics	
Fever (≥37.5 °C)	8 (80%)
- Maximum temperature: mean (range) (SD)	38.0 °C (37.6–38.4) (0.2)
- Overall duration: mean (range) (SD)	2.6 days (1–4) (0.9)
Cough	
- Productive	7 (70%)
- Dry	2 (20%)
Sore throat	6 (60%)
Running nose	6 (60%)
Blocked nose	4 (40%)
Myalgia	4 (40%)
Arthralgia	0 (0%)
Shortness of breath	0 (0%)
Asymptomatic	1 (10%)[a]
Total duration of illness: mean (range) (SD)	7.4 days (3–14) (3.2)
Complications	
Pneumonia	0 (0%)
Hospitalization	1 (10%)

[a] Includes asthma, ischaemic heart disease, diabetes mellitus, hyperlipidemia, malignancy, immunosuppressed state, any other chronic cardiac, pulmonary, renal or liver condition.

4. Results

From June 22 to July 30, 2009, 1032 personnel received oseltamivir prophylaxis, and 10 cases (1%) of laboratory-confirmed prophylaxis failures were detected. The entire cohort comprised of males with a mean age of 22.3 years and mean BMI of 21.8 kg/m^2. The mean age of the cases was 22.3 years. The average BMI of the cases was 24.3 kg/m^2 – 2 were obese (BMI ≥ 27.5), 3 were overweight (BMI 23.5–27.4) and 1 was underweight (BMI < 18.5). One patient with hyperlipidemia was on dietary control (Table 1).

4.1. Clinical characteristics and complications

Of the 10 cases, 1 case was asymptomatic throughout and was identified through our active surveillance initiative (in the first three outbreaks, thrice weekly surveillance nasopharyngeal swabs of the affected cohorts were carried out until no further cases were detected). Eight cases had fever with a mean temperature of 38.0 °C, and 9 cases had cough of which 7 (70%) cases were productive. There were no severe complications – 1 individual had mild illness but chose hospitalization to isolate himself from his family.

The mean duration of exposure before starting oseltamivir was 1.9 days (SD 0.9), while the mean duration of oseltamivir consumption before symptom onset was 1.9 days (SD 1.4) (Table 2). All cases were compliant to prophylaxis. Post-initiation of prophylaxis, two cases had nausea and vomiting, and 1 each had headache, dizziness, diarrhea, and rash respectively; these manifestations could either

Table 2
Time course of oseltamivir commencement and symptom onset in the 10 cases of prophylaxis failure.

	Days of exposure before starting oseltamivir	Days of oseltamivir prophylaxis completed before symptom onset
Serviceman 1	3	5
Serviceman 2	3	4[a]
Serviceman 3	1	2
Serviceman 4	2	1
Serviceman 5	2	1
Serviceman 6	1	3
Serviceman 7	1	1
Serviceman 8	1	2
Serviceman 9	3	1
Serviceman 10	2	1

[a] Serviceman was asymptomatic. Date of diagnosis was taken to be date of symptom onset as nasopharyngeal swab 2 days prior was negative.

be due to the influenza infection or oseltamivir side effects. There were no severe adverse or neuropsychiatric side effects.

4.2. Resistance testing and molecular sequencing

Nine cases had sufficient RNA material for whole genome sequencing. As oseltamivir directly targets the sialic acid binding pocket in the neuraminidase protein (NA), we investigated possible mutations in this region. The well-studied mutations in position 275 are known to convey resistance. In the 9 samples tested, none had the mutated form encoding tyrosine, which would cause resistance, while 7 gave clear evidence of the drug-sensitive wildtype histidine (H275) (sequences from the other two samples were suboptimal at the specific region). All 9 samples did not reveal mutations in another previously known position affecting oseltamivir resistance in N2 neuraminidases, position 119, with the encoded protein showed drug-sensitive glutamate.

To systematically address if new resistance mutations could have occurred, 35 positions near the drug-binding pocket were screened (supplementary Table 1). We obtained reliable amino acid sequences with >90% coverage over all samples, and showed that drug-sensitive wildtype residues could be confirmed with no mutations found except in one case. The only identified mutation was E47G, found in the neuraminidase protein. Although this mutation would have changed the local electrostatic environment by removing a negative charge, its position in the non-globular stalk is far from the drug-binding pocket and thus unlikely to affect drug binding significantly.

Fig. 1. Identified mutations (red balls) mapped to 3D structural model of viral hemagglutinin (grey ribbons) in complex with the host cell receptor (blue balls), based on PDB template 1RVT. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

Table 3 shows the identified mutations. The most frequently identified mutation, HA T220S, is located at the HA head domain (Fig. 1), which has the important function of host cell recognition. However, the mutation's orientation facing away from the binding site would minimize potential effects on viral biology. Serine at position 220 is common among seasonal H1N1 strains and no clinical abnormalities for either serine or threonine at position 220 have been found. HA E275V and HA T333A appear at neutral positions and also globally without clinical abnormalities. We also report a HA D239G mutation. Position 239 on top of the receptor at the sugar binding pocket (Fig. 1) likely influences host cell sialic acid binding.

Mutations K660R and L607V in the viral polymerase protein PB2 occur in its C-terminal domain which is implicated in binding the human host protein importin alpha which plays a role in nuclear import. Neither appears close enough to affect the respective interaction (Fig. 2). Another mutation, PB2 V292I, is located just before the CAP-binding domain in a region without known structure. V292I and K660R had been previously found in several patients without reported anomalies.[21]

Mutation F103S in the viral non-structural protein 1 (NS1) appears in a position critical for interaction with the F2F3 fragment of human cellular factor CPSF30 (Fig. 3), which is implicated in suppressing host antiviral response.[22] As the wildtype aromatic phenylalanine fits into a hydrophobic pocket of the human protein, mutation to the small and polar serine would definitely weaken this interaction, possibly suggesting a reduction in virulence. Most occurrences of this mutation globally were reported from Singaporean samples,[21] suggesting local transmission chains.

Mutation W104G in the viral nucleoprotein (NP) replaces a buried hydrophobic residue with a flexible non-hydrophobic

Table 3
Summary of identified mutations, their expected molecular effects and global occurrences.

Protein	Mutation[a] (*alternative numbering*)	Number of occurrences	Expected molecular effect	Global wild type occurrences	Global mutant type occurrences	Global other amino acid occurrences
HA	T220S *(H3: 206, H1: 203)*	6	Neutral, common variation	1013(56.62%)	774(43.26%)	2(0.11%)
PB2	K660R	4	Neutral	1024(94.90%)	55(5.10%)	0(0.00%)
NS1	F103S	3	Altered host protein interaction	1092(97.59%)	25(2.23%)	2(0.18%)
PB2	L607I	2	Neutral	1086(100.00%)	0(0.00%)	0(0.00%)
PB2	V292I	1	Neutral	1041(97.93%)	22(2.07%)	0(0.00%)
HA	D239G *(H3:225, H1: 222)*	1	Altered host cell receptor interaction	1650(92.80%)	24(1.35%)	104(5.85%)
HA	E275V *(H3: 261, H1: 258)*	1	Neutral	1757(99.55%)	6(0.34%)	2(0.11%)
HA	T333A *(H3: 318, H1: 316)*	1	Neutral	1738(99.89%)	1(0.06%)	1(0.06%)
NP	W104G	1	Affect viral protein stability	1147(100.00%)	0(0.00%)	0(0.00%)
NA	E47G *(N2: 51, N1: 47)*	1	Neutral, in stalk far from drug binding site	1416(99.86%)	2(0.14%)	0(0.00%)

[a] The numbering and amino acid changes are relative to early representative strain A/New York/20/2009(H1N1) to which most samples of this study were most closely related (minimum number of mutations). Alternative HA and NA numbering are in relation to seasonal H3N2 (A/Brisbane/10/2007(H3N2)) or H1N1 (A/Puerto Rico/8/1934(H1N1)) residue positions.

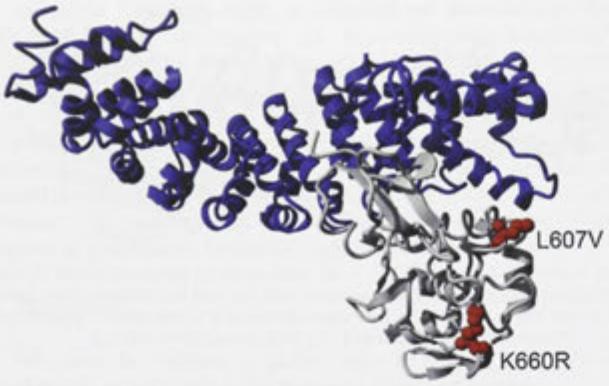

Fig. 2. Identified mutations (red balls) mapped to 3D structural model of viral PB2 (grey ribbons) in complex with the host protein importin alpha (blue ribbons), based on PDB templates 3CW4 and 2JDQ. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

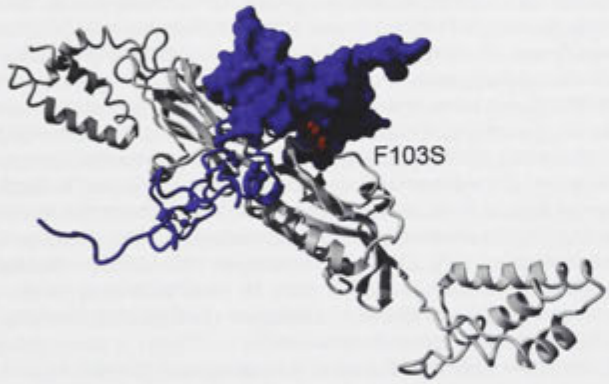

Fig. 3. Identified mutation (red balls) mapped to 3D structural model of viral NS1 (dimer: both units as grey ribbons) in complex with the host protein human cellular factor CPSF30 (dimer: 1 unit as blue ribbons and 1 unit in blue surface representation), based on PDB templates 3EU6 and 2RHK. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

glycine (Fig. 4) which may destabilize the surrounding structural scaffold. As structural integrity of NP is required for viral RNA genome binding and organization, the W104G mutation could attenuate viral replication efficiency.

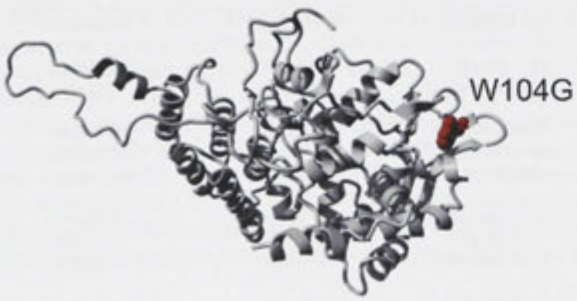

Fig. 4. Identified mutation (red balls) mapped to 3D structural model of viral nucleoprotein (grey ribbons), based on PDB template 2Q06. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

5. Discussion

Oseltamivir has been used for prophylaxis against influenza, and cases of prophylaxis failures have been documented.[3–6] Confirmed prophylaxis failures accounted for about 1% (10/1032) of our cohort, comparable to previous studies of 1.2%[5] and 1.4%.[3] Although subclinical cases could have been missed, this is lower than the 44% infection rate determined through serological testing in a similar group of servicemen not given oseltamivir prophylaxis in a separate study.[23,24]

Prophylaxis failures can occur secondary to antiviral resistance.[7,8,11] However, to date, the pandemic influenza A (H1N1-2009) virus remains largely sensitive to oseltamivir with about 200 reported cases of oseltamivir resistance – all showing H275Y mutation and thus remaining sensitive to zanamivir.[10] None of the prophylaxis failure cases in our study had this H275Y mutation. There have also been no wide community circulation of oseltamivir resistant viruses except for the transmission of resistant viruses in a few local settings.[10,11] Primary prophylaxis failures not due to mutations that confer resistance are also a common occurrence.[3,4] In our study, none of the prophylaxis failures had the H275Y or other mutations that may have produced antiviral resistance.

Primary prophylaxis failures may be due to longer exposure durations before prophylaxis commencement, insufficient time for prophylaxis effect, or insufficient dosing. The mean duration of exposure before starting oseltamivir was 1.9 days (SD 0.9) with more than half having been exposed for 2 days or longer. This exposure duration may have allowed substantial viral replication before prophylaxis, thereby reducing its efficacy. The mean duration of oseltamivir consumption before symptom onset was 1.9 days (SD 1.4), with about half having taken only 1 dose of oseltamivir – possibly resulting in insufficient time for therapeutic dosing. Half of our prophylaxis failure cases were overweight/obese, compared with <10% for the general military population, raising the possibility of inadequate dosing since the effective dose per body weight would be reduced. Although a previous study showed no significant difference between once compared to twice daily dosing of oseltamivir prophylaxis,[5] this requires further investigation.

From the genome sequencing and 3D simulations, none of the highlighted mutations were expected to interfere with prophylaxis through direct effects on oseltamivir binding. Only mutation D239G in HA has the potential to increase virulence as a hypothetical mechanism in developing symptoms despite antiviral prophylaxis. The HA D239G mutation has been identified in fatal or severe cases[10] in additional to mild cases; we report herein the first case with this mutation in Singapore, which was a mild case. The importance of position 239 for host cell recognition and host specificity was highlighted previously as part of a double mutation (E204D, G239D) that turned the viral hemagglutinin from avian to human specificity, although position 204 appeared to be the stronger determinant.[25] G at position 239 should, therefore, favor the avian α-2,3 receptor type which is also found on specialized human cells. It cannot be excluded that early prophylaxis could have eventually weakened the effects of the potentially dangerous D239G mutation since our case did not display any severe complications.

Although these 10 individuals failed prophylaxis, the prophylaxis regime may have conferred some protection against severe illness as they had mild illness, similar to influenza cases with early treatment with oseltamivir.[26,27] Due to the small sample size, further cohort studies with comparison groups are needed to determine if primary prophylaxis failures are less symptomatic and have less complications compared to cases not on prophylaxis, as well as further delineate the reasons for prophylaxis failures. Addi-

tional experimental phenotypic testing should also be considered in future studies to understand the link between genetic sequences and resistance.

We have shown that primary prophylaxis failures for pandemic influenza A (H1N1-2009) occur. None of these were due to the H275Y mutation and were unlikely to be caused by other viral mutations based on genetic sequences.

Conflict of interest

VJL has received research support from GSK. No other conflict of interest exist.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jcv.2010.10.004.

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Supplementary Table 1. Detailed sequencing of positions close to drug-binding pocket

Neuraminidase Distance to drug	N2 pos	N1 pos	ref (NY 20)	Service-man 1	Service-man 2	Service-man 3	Service-man 4	Service-man 5	Service-man 6	Service-man 7	Service-man 8	Service-man 9	%AA coverage
<5 A	118	118	R	R	R	R	R	R	R	R	R	R	100.0%
<5 A	119	119	E	E	E	E	E	E	E	E	E	E	100.0%
<5 A	134	134	L	L	L	L	L	L	L	-	L	L	88.8%
<5 A	151	151	D	D	D	D	D	D	D	D	D	D	100.0%
<5 A	152	152	R	R	R	R	R	R	R	R	R	R	100.0%
<5 A	156	156	R	R	R	R	R	R	R	R	R	R	100.0%
<5 A	178	179	W	W	W	W	W	W	W	W	W	W	100.0%
<5 A	179	180	S	S	S	S	S	S	S	S	S	S	100.0%
<5 A	221	222	N	N	N	N	N	N	N	N	N	N	100.0%
<5 A	222	223	I	I	I	I	I	I	I	I	I	I	100.0%
<5 A	223	224	L	L	L	L	L	L	L	L	L	L	100.0%
<5 A	224	225	R	R	R	R	R	R	R	R	R	R	100.0%
<5 A	225	226	T	T	T	T	T	T	T	T	T	T	100.0%
<5 A	227	228	E	E	E	E	E	E	E	E	E	E	100.0%
<5 A	246	247	S	-	-	S	S	S	S	S	S	-	66.7%
<5 A	274	275	H	H	-	H	H	H	H	H	-	H	77.8%
<5 A	276	277	E	E	E	E	E	E	E	E	-	E	88.9%
<5 A	277	278	E	E	E	E	E	E	E	E	E	-	88.9%
<5 A	292	293	R	R	-	R	R	R	R	R	R	-	77.8%
<5 A	294	295	N	N	N	N	N	N	N	N	N	N	100.0%
<5 A	348	345	G	G	G	G	G	G	G	G	G	G	100.0%
<5 A	349	346	V	V	V	V	V	V	V	V	V	V	100.0%
<5 A	371	368	R	R	-	R	R	R	R	R	R	R	88.9%
<5 A	406	402	Y	Y	-	Y	Y	Y	Y	-	Y	-	66.7%
			%AA cover-age	95.80%	79.20%	100.00%	100.00%	100.00%	100.00%	91.70%	91.70%	83.30%	
>5 A <7 A but highly conserved	136	136	Q	Q	Q	Q	Q	Q	Q	-	Q	Q	88.9%
>5 A <7 A but highly conserved	201	202	A	A	-	A	A	A	A	-	A	A	77.8%
>5 A <7 A but	242	243	T	T	-	T	T	T	T	T	T	-	77.8%

highly conserved >5 A <7 A but highly conserved	243	244	D	-	-	D	D	D	D	D	D	D	77.8%
highly conserved >5 A <7 A but highly conserved	244	245	G	G	-	G	G	G	G	G	-	-	66.7%
highly conserved >5 A <7 A but highly conserved	250	251	A	A	A	A	A	A	A	A	A	-	88.9%
highly conserved >5 A <7 A but highly conserved	293	294	D	D	-	D	D	D	D	D	D	D	88.9%
highly conserved >5 A <7 A but highly conserved	295	296	W	W	W	W	W	W	W	W	W	W	100.0%
highly conserved >5 A <7 A but highly conserved	350	347	K	-	-	K	K	K	K	K	K	K	77.8%
highly conserved >5 A <7 A but highly conserved	405	401	G	G	G	G	G	G	G	G	G	G	100.0%
highly conserved >5 A <7 A but highly conserved	425	425	E	E	-	E	E	E	E	E	E	E	88.9%
			%AA cover-age	81.8%	36.3%	100.0%	100.0%	100.0%	100.0%	81.8%	90.9%	72.7%	
			total % AA	91.4%	65.7%	100.0%	100.0%	100.0%	100.0%	88.6%	91.4%	80.0%	
											Average overall coverage:		**90.8%**

Chapter Twelve

Effectiveness of Combinations of Public Health Measures in Reducing the Spread of Influenza

The previous chapters have described the possible cross-reactivity of seasonal vaccination against the 2009 pandemic influenza strain, and the effectiveness of anti-viral drugs as prophylaxis. However, the uptake of seasonal influenza vaccine alone is unlikely to be sufficient to avert the pandemic's spread, and pandemic vaccines, whilst likely to be effective, are also unlikely to be available ahead of the first pandemic wave in any sufficient numbers. Anti-viral drugs, while useful in specific settings, are not a feasible solution to prevent the spread of influenza across the world.

Public health measures may therefore be the only set of strategies that are available to reduce the spread of influenza in most populations. As discussed in Chapter Seven, most of the available suggestions for the effectiveness of public health measures to reduce the spread of influenza are from mathematical modeling studies. There have been few opportunities to study the effect of public health measures in sizeable populations due to the difficulty in collecting sufficient data on infection rates in separate groups where different interventions have been used.

The next and final manuscript in this thesis provides much awaited evidence on the effectiveness of public health measures in reducing the spread of influenza. This is a detailed analysis of the military cohort which was part of the study described in Chapter Five. The Singapore military provided the ideal opportunity to study these measures due to the well-circumscribed groups with different sets of measures implemented, as well as the seroepidemiological study design which allows all probable cases to be identified in addition to clinical cases. One of the cohorts selected for this study was healthcare workers, which may be affected by the contact with infected patients, and the use of infection control measures together with health

education. It will therefore be important to determine if measures used for healthcare workers are effective in reducing their risk of infection.

The results provide conclusive evidence on the effectiveness of combinations of public health measures in reducing the spread of influenza. In the healthcare worker cohort, these measures are likely to have reduced the workplace exposure, but the overall training in infection control and hygiene procedures may have also influenced their behavior outside of the workplace to result in an additional overall reduction in transmission.

Study 10

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Effectiveness of Public Health Measures in Mitigating Pandemic Influenza Spread: A Prospective Sero-Epidemiological Cohort Study

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Background. Few studies have validated the effectiveness of public health interventions in reducing influenza spread in real-life settings. We aim to validate these measures used during the 2009 pandemic.

Methods. From 22 June to 9 October 2009, we performed a prospective observational cohort study using paired serum samples and symptom review among 3 groups of Singapore military personnel. "Normal" units were subjected to prevailing pandemic response policies. "Essential" units and health care workers had additional public health interventions (eg, enhanced surveillance with isolation, segregation, personal protective equipment). Samples were tested by hemagglutination inhibition; the principal outcome was seroconversion to 2009 influenza A(H1N1).

Results. In total, 1015 individuals in 14 units completed the study, with 29% overall seroconversion. Seroconversion among essential units (17%) and health care workers (11%) was significantly lower than that in normal units (44%) ($P < .001$). Symptomatic illness attributable to influenza was also lower in essential units (5%) and health care workers (2%) than in normal units (12%) ($P = .06$). Adjusted for confounders, unit type was the only significant variable influencing overall seroconversion ($P < .05$). From multivariate analysis within each unit, age ($P < .001$) and baseline antibody titer ($P = .012$) were inversely related to seroconversion risk.

Conclusions. Public health measures are effective in limiting influenza transmission in closed environments.

The 2009 influenza A(H1N1) pandemic affected most countries within months of its emergence. Despite early identification of the virus and massive scale-up of vaccine production, initial responses were largely based on pre-existing pandemic plans due to the pandemic's rapid spread before the vaccine's availability months later. The use of combination strategies, including anti-viral treatment of cases, prophylaxis and quarantine of close contacts, and community social distancing have been shown in computational mathematical models to reduce influenza attack rates [1–3]. Although policy makers worldwide have adopted these interventions, there are few studies that have clearly validated these models' findings for pandemic influenza control in real-life settings, which is critical to assess their actual effectiveness [4].

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Influenza pandemic attack rates have been higher than those of nonpandemic influenza [5], whereas attack rates have also been high in environments such as military facilities (42%–58%) [6, 7] and boarding schools (71%) [8], even in nonpandemic years. There is indirect evidence that schools are potential amplifying arenas for influenza transmission [9, 10], and the same may also be true for other closed and semiclosed environments. Notably, the first outbreaks of 2009 influenza A(H1N1) in Singapore, a tropical city-state in Southeast Asia, occurred in such settings: a tertiary education institute, army camps, and church camps [11]. Therefore, measures that can control outbreaks early in closed or semiclosed environments may be an important pandemic mitigation strategy.

Most population-based studies of nonpharmacological interventions have been based on influenza-like illness (ILI) or laboratory-confirmed influenza [12, 13], but there are difficulties arising from lack of specificity of clinical definitions and documentation of laboratory-confirmed influenza. Sero-epidemiological studies are more definitive in determining population infection rates and effectiveness of interventions [14, 15], yielding critical information on preventive measures that cannot be obtained from observational studies alone. Previous pandemic studies have either relied on observational data [16, 17], single post-pandemic blood specimens [18], or cross-sectional serological samples [19]. However, the presence of cross-reactive antibodies to 2009 influenza A(H1N1) [19, 20] underscores the importance of using paired samples for precise measurement of seroconversion rates.

We therefore undertook a prospective observational study using paired serum samples among 3 distinct groups of military soldiers in Singapore to evaluate the impact of public health measures for the control of pandemic influenza before the pandemic vaccine was made available.

METHODS

This study was performed on the Singapore military from 22 June to 9 October 2009 and was part of a larger seroepidemiological investigation involving other cohorts—community adults, health care workers from 1 hospital, and long-term care facility residents [21]. Singapore is a globally connected tropical city-state, and the Singapore military is composed of both conscript personnel, where all men enlist after high school, and regular employees. These personnel work and reside in military camps during weekdays and return to the community on weekends, resulting in a mostly closed-living environment but with regular exposure to the general community.

The military's pandemic response plan was developed prior to the pandemic and involved a stratified and targeted response to ensure operational readiness. "Normal" units followed the prevailing national pandemic response policies, in which individuals were provided general health education on respiratory and hand hygiene and were advised to seek medical care if ill.

"Essential" units, defined as those units critical to the military's functioning where absenteeism at any time point must be minimized, received an additional set of public health measures during the epidemic's duration. This included enhanced surveillance (daily recorded temperature and symptom monitoring with prompt identification and reporting of acute respiratory illnesses [ARIs]) with medical referral and provision of home medical leave for these cases, as well as segregation of units into smaller working subgroups. Segregation as a form of social distancing entailed noncontact when possible between subgroups of as small as 20 individuals, including having different activity and meal times, and times of entry and exit from camp. Health care workers in military medical centers were subjected to similar enhanced surveillance measures as essential units and in addition wore N95 masks, gloves, and gowns continuously during their working hours; compliance was ensured through regular inspections. Both these groups also received routine annual seasonal influenza vaccination, which was not routinely offered to normal units, who were, however, free to obtain this on their own.

These 3 main groups were selected a priori to evaluate the effectiveness of the different levels of interventions summarized in Table 1. Multiple units representative of each main group were chosen, and participants were recruited from within these units. The working and living facilities, and intraunit interactions, were generally similar across the units.

Three blood samples were collected from each participant. The first sample (sample A) was taken from 22 June to 1 July 2009, immediately following the appearance of local community transmission in Singapore in the second half of June 2009 [22], before the widespread epidemic. None of the units selected had any recorded increase in respiratory illness or had any confirmed 2009 influenza A(H1N1) cases before sampling. The second sample (sample B) was mostly taken from 20 August to 3 September 2009, 3–4 weeks after the epidemic's peak in Singapore, which occurred during the first week of August 2009 [23, 24]. The final postepidemic sample (sample C) was taken from 29 September to 9 October 2009, 4–5 weeks after epidemic activity had subsided and national ARI and ILI rates had returned to baseline levels [23, 24].

Standardized questionnaires were given to participants during the 3 blood samplings, and at 3-week intervals in between. The questionnaires collected data on demographics, medical history, any previous vaccination history, and new onset symptoms related to influenza. ARI was defined as onset of rhinorrhea, nasal congestion, sore throat or cough; febrile respiratory illness (FRI) was defined as ARI with concurrent self-reported fever or temperature of $\geq 37.5^{\circ}C$ [25]. Written informed consent was obtained, and study approval was granted by the mil-

Table 1. Characteristics of Study Population

Characteristic	Overall	Normal personnel	Essential personnel	Health care workers
No. of units in each cohort	14	5	5	4
Total no. of individuals in the units	1515	594	757	164
No. of individuals who participated	1166 (77)	472 (79)	567 (75)	127 (77)
Mean sample size who participated per unit (SE)	73 (11)	87 (4)	94 (21)	27 (7)
Samples				
At least 2 samples provided	1015/1166 (87)	437/472 (93)	470/567 (83)	108/127 (85)
Interventions		Standard pandemic plan	Standard pandemic plan plus	Standard pandemic plan plus
			• Segregation among working subgroups	• PPE, including N-95 during working hours
			• Enhanced surveillance and isolation[a]	• Enhanced surveillance and isolation [a]
			• Seasonal influenza vaccination	• Seasonal influenza vaccination
Demographics				
Median age[b]	20	19	22	21
IQR	19–22	18–20	20–26	20–22
Range	17–61	17–51	18–61	18–54

NOTE. Data are no. (%) of participants, unless otherwise indicated. IQR, interquartile range; PPE, personal protective equipment; SE, standard error.

[a] Included daily temperature monitoring and prompt reporting of cases of acute respiratory illness, with provision of home medical leave.

[b] Significant at the $P < .001$ level.

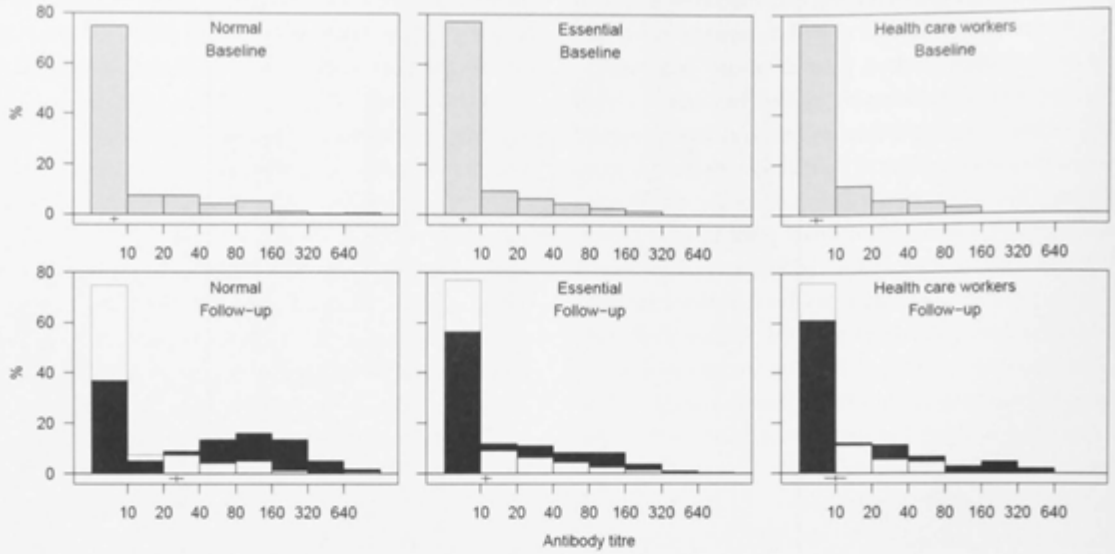

Figure 1. Proportion of participants with the respective baseline and follow-up antibody titers among the 3 groups. For the follow-up graphs, the black bars are the follow-up antibody titers, whereas the white bars are the baseline titers superimposed for comparison. Geometric mean titers are shown in the row below the bars, with the mean value indicated by the vertical line and 95% confidence interval indicated by the horizontal line.

itary's Joint Medical Committee for Research and the Australian National University's ethics review board.

Laboratory methods. For each sampling, 5–10 mL of venous blood was taken. The hemagglutination inhibition assay was performed for all samples in parallel, according to standard protocols [26] at the World Health Organization (WHO) Collaborating Centre for Reference and Research for Influenza in Melbourne, Australia.

The serum was pretreated with receptor destroying enzyme (RDE [II]; Deka Seiken Co Ltd), 1:4 (vol/vol), at 37°C for 16 h; then the enzyme was inactivated by addition of an equal volume of 1.5% tri-sodium citrate (Ajax Chemicals) and incubation at 56°C for 30 min. Egg-grown A/California/7/2009 A(H1N1–2009) virus was purified by sucrose gradient, concentrated, and inactivated with β-propiolactone, to create an influenza zonal pool (IZP) preparation. In total, 25 μL of (4HAU) IZP-A/California/7/2009 virus was incubated at room temperature with an equal volume of RDE-treated serum. Serum samples were titrated in 2-fold dilutions in phosphate-buffered saline from 1:10 to 1:1280. Following 1 h incubation, 25 μL of 1% (vol/vol) turkey red blood cells was added to each well. Hemagglutination inhibition was read after 30 minutes. Titers were expressed as the reciprocal of the highest dilution of serum where hemagglutination was prevented.

To determine seroconversion to 2009 influenza A(H1N1) in individual participants, we compared antibody titers between successive pairs of blood specimens. Seroconversion was defined as a $\geq$4-fold rise in antibody titers [27].

Statistical analysis. The principal outcome measure was seroconversion to 2009 influenza A(H1N1). The secondary outcome measure was the presence of ARI and/or FRI likely due to influenza (ie, symptomatic seroconversions). To determine the sample size, we assumed 25% seroconversion in normal units on the basis of previous studies and a difference with the intervention groups of at least 10%. To achieve 80% statistical power at the 5% significance level for pairwise comparisons

Table 2. Rates of Seroconversion and Symptomatic Seroconversion among the 3 Groups

	Seroconversion			Symptomatic seroconversion, mean proportion (SE)[b]
Unit type	Mean proportion (SE)	Relative risk (95% CI)[a]	*P* value[a]	
Normal	44 (3)	1	N/A	12 (3)
Essential	17 (3)	0.39 (0.26–0.54)	<.001	5 (3)
Health care worker	11 (3)	0.26 (0.07–0.46)	<.001	2 (3)

NOTE. CI, confidence interval; N/A, not applicable; PPE, personal protective equipment; SE, standard error.

[a] Comparing the intervention cohorts (essential cohort or health care cohort) to the nonintervention normal cohort.

[b] $P = .061$, comparing all 3 groups using the Kruskal-Wallis test.

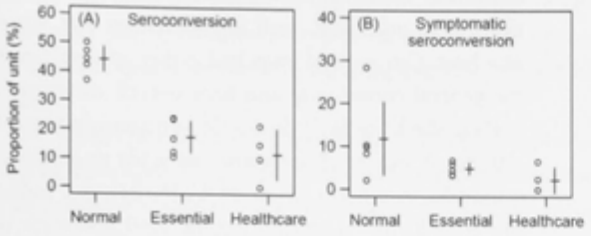

Figure 2. Rates of seroconversion (*A*) and symptomatic seroconversion (*B*) within individual units among the 3 groups.

would require 250 independent participants per group. At the unit level, a postulated mean difference of 10% and 5% standard deviation require 5 units to attain the same power and significance level. We therefore aimed for 250 individuals in each comparison group, spread among 5 units per group. With dependence of final outcomes, sample sizes would have to increase; however, logistical constraints resulted in recruitment of more normal and essential soldiers but fewer health care workers.

Demographics were compared using the Kruskal-Wallis test for age and Pearson χ^2 test for sex, using the Hope Monte Carlo version for small sample sizes with 1 million iterations [28]. The Pearson χ^2 test was also used to assess whether baseline titers varied by seasonal vaccination status. We also computed the geometric mean titers (GMTs) for the 3 groups, where titers <10 were assigned a value of 5.

Other statistical analyses were based on the dependent nature of disease status within units due to the contagious nature of influenza. For the main analysis, we therefore treated the military unit as the basic statistical unit of interest, implicitly assuming that individual military units are effectively independent—their members working and living separately from other military units. Overall unit-level seroconversion rates in the 3 groups were compared using a Fisher analysis of variance (ANOVA) test with Gaussianness tested by the Shapiro-Wilk test [29] and homoskedasticity by the Bartlett test [30]. Confidence intervals (CIs) for relative risks (RRs) were calculated using the Fieller method [31].

Finally, we performed 2 multivariate analyses. The first was an ecological study on unit seroconversion using properties of the unit as predictors, namely, mean age, proportion of men, and antibody titer at baseline, within a linear model framework to assess whether these confounded unit type. In addition, an individual-level multiple logistic regression was performed using age, sex, titer at baseline, and vaccine status (the only intervention component that could be separately quantified) as predictors along with a unit-specific dummy variable, allowing the possible effect of these covariates to be assessed, conditioned on attack rates within each unit. All analyses were performed with R software programming language [32].

RESULTS

Study population. A total of 1515 individuals from 14 different military units were initially selected. Of these, 1166 (77%) agreed to participate in the study, and 87% (1015/1166) of participants completed the study by providing a baseline and at least 1 other sample for paired sample serological analysis. The majority who did not complete the study were soldiers who had left the military during the study. The follow-up rates for the normal, essential, and health care worker cohorts were 93% (437/472), 83% (470/567), and 85% (108/127), respectively.

Table 1 shows the general characteristics of the cohort. The majority of the participants were young men, reflecting the general military population. Essential units had a significantly higher proportion of personnel of older ages compared with the other groups ($P < .001$). Health care worker units were smaller than the other units because these were teams working in primary health care facilities that had fewer personnel than other training or working units.

Primary and secondary outcomes. The baseline and follow-up antibody titers among the 3 groups are shown in Figure 1. There was no significant difference in the baseline titers among the 3 groups, whereas the GMT on follow-up were significantly higher in normal personnel than in the other cohorts ($P < .001$). The mean fold increase in titers comparing baseline to follow-up titers for each individual was 1.65 (95% CI, 1.53–1.77) for essential personnel, 1.52 (95% CI, 1.30–1.73) for health care workers, and 2.72 (95% CI, 2.52–2.91) for normal personnel ($P < .001$).

Overall, the primary outcome of serologically confirmed 2009 influenza A(H1N1) infection was 29% (295/1015). Mean serologically confirmed infections were significantly lower in both essential worker units (17%; $P < .001$) and health care worker units (11%, $P < .001$) than in the normal units (44%) (Table 2). However, no significant difference was found between essential units and health care worker units (RR, 0.66; 95% CI, 0.14–1.54; $P = .22$). The secondary end point of symptomatic illness attributable to influenza infection was lower among essential units (4.8%) and health care workers (2.2%) than in normal units (12%) and of borderline statistical significance ($P = .061$). Figure 2 shows the rates of seroconversion and symptomatic seroconversion within individual units among the 3 cohorts, whereas the exact breakdown by individual units can be found in Table 3.

Performing a multivariate, ecological, unit-level analysis with unit type, proportion of men, mean age, and baseline antibody

Table 3. Rates of Seroconversion and Symptomatic Seroconversion in Each Individual Unit

This table is available in its entirety in the online version of *Journal of Infectious Diseases*.

Table 4. Multivariate Logistic Regression of Factors Affecting Seroconversion within Each Individual Unit

Predictor	OR	95% CI	*P* value
Age, years	0.86	(0.80–0.94)	<.001
Sex, men relative to women	2.73	(0.56–13.23)	0.21
Seasonal influenza vaccine	1.03	(0.63–1.68)	0.89
Baseline titer	0.80	(0.68–0.95)	.012

NOTE. CI, confidence interval; OR, odds ratio.

titers against 2009 influenza A(H1N1) as predictors of proportion who seroconverted, we found that only unit type was a significant predictor, with both essential units and health care units having lower proportions infected than did normal units (essential vs normal, $P = .007$; health care vs normal, $P = .001$). Age ($P = .59$), sex ($P = .89$), and baseline titer ($P = .74$) had no effect at this scale. This suggests that although age and sex patterns in the units differ, these differences did not result in different seroconversion rates other than those attributable to the associated interventions themselves.

To assess whether age, sex, or baseline antibody titers had any protective effect at the individual level, we performed a multiple logistic regression with a separate intercept parameter per unit. Seasonal vaccine status, the only intervention we could separately quantify, was also included as a parameter. Table 4 summarizes this multivariate analysis and the corresponding odds ratios. Age ($P < .001$) and initial titer ($P = .012$) were significantly inversely related to the risk of seroconversion after accounting for different exposures in the different units, although there was no evidence of effect on the basis of sex ($P = .21$). Seasonal vaccine at the individual level was also not a significant predictor of individual seroconversion ($P = .89$). In addition, there was no significant difference in baseline geometric mean antibody titers (GMTs) between those who received previous seasonal vaccine and those without (11.2 vs 15.1; $P = .15$).

Epidemic curves. Figure 3 shows the cumulative clinical epidemic curves based on reported symptom onset of ARI and/or FRI among those who seroconverted, stratified by unit type and compared to the estimated community cumulative epidemic curve [24]. The peak of the epidemic for normal units occurred earlier than in the essential units, health care worker units, and the general community.

CONCLUSIONS

This is one of the first large serological cohort studies to document the effectiveness of combined public health interventions against pandemic influenza. These interventions can be performed with minimal disruption of essential services and potentially reduce the impact of influenza illness and transmission, leading to lower peak infection rates and point absenteeism. Delaying disease spread may allow other interventions to be instituted, such as vaccination that was only available later. Our normal units had earlier epidemic peak than did the general community and high overall seroconversion rates (44%), which are likely due to the younger adult ages and closed settings. A similar observation was made in seasonal influenza outbreaks in schools (21%–71%) [8, 33] and military camps (42%–58%) [6, 7], and during the 2009 pandemic with >30% attack rate during a school outbreak [34]. It is therefore important to reduce influenza transmission in similar closed settings where high attack rates during a short time period and high absenteeism are undesirable, including schools, boarding facilities, long-term elderly care facilities, and essential services such as health care workers. This may also reduce the chance of such settings acting as amplifiers for a novel virus [9, 10, 35].

Our study showed the likely effectiveness of public health measures, in particular, enhanced surveillance (daily temperature taking and prompt ARI reporting) with isolation through home medical leave, and segregation of smaller subgroups, on the spread of influenza. These measures were effective, easy to administer, and sustainable during the entire 2-month epidemic. Symptomatic seroconversion was also reduced in the intervention cohorts with marginal significance, suggesting that the interventions proportionally reduced symptomatic cases (a proxy for absenteeism). Although seasonal influenza vaccination may elicit cross-reactive antibodies against the pandemic strain [20], there was no evidence in our study that individuals vaccinated against seasonal influenza had lower risk of infection above the other interventions. Essential units had delayed onset compared

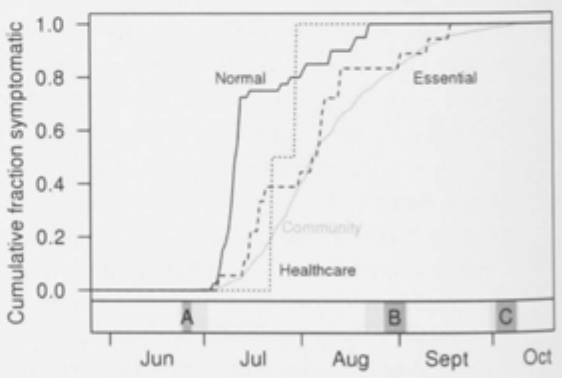

Figure 3. Cumulative clinical epidemic curves among those who seroconverted for each group, compared with estimated community cumulative epidemic curve from GP sentinels. Colored bands indicate sampling times, with dark bands representing interquartile range (IQR) and light bands representing range (excluding 11 samples taken from 9 to 10 September 2009). Multiple acute respiratory illness and/or febrile respiratory illness episodes per individual were proportioned equally and attributed to that individual.

with normal units but similar to that of the community (Figure 3), possibly due to community exposures and the effectiveness of the public health measures in delaying spread in the military setting.

Our results provide serological evidence to support previous observational studies during pandemics where public health measures designed to reduce transmission such as social distancing and restrictions on public gatherings, and isolation and quarantine significantly reduced overall mortality in the absence of an efficacious vaccine [16, 17, 36]. The measures used in our study were minimally disruptive and ensured business continuity by minimizing peak infection rates and point absenteeism and can be similarly applied to other closed settings over long durations with prior planning. For example, in schools, daily temperature taking and symptom monitoring can be implemented on entry, and anyone with respiratory illnesses can be referred for medical consult. In addition, social distancing can be achieved through students being segregated by classrooms or educational levels with staggered entry and exit, breaks and meal times, and deferment of school-wide mass gatherings. These measures can potentially reduce simultaneous spread across classes, without the need for disruptive school closures.

Health care workers had reduced attack rates compared with normal units possibly due to the use of personal protective equipment, including wearing of N-95 masks during working hours, on top of the enhanced surveillance. Health care workers also had lower seroconversion rates compared with essential units, although this was not statistically significant (RR, 0.66; $P = .22$). At the same time, 11% of health care workers showed serological evidence of infection, highlighting the possible role played by nonoccupational acquisition of influenza. Although health care staff would have similar infection risks from settings outside their work environment, they might reasonably be expected to have higher occupational exposure to pandemic influenza cases and thus would have been expected to have had higher infection risks. The combination of personal protective equipment, together with enhanced surveillance, may have reduced seroconversion rates among health care workers despite their higher risk exposure. These strategies may be similarly applied to health care workers in other settings, reducing their risk of infection and minimizing disruption to the critical provision of health care.

We have also found that age and baseline antibody titers were independently inversely correlated with seroconversion. This is consistent with other observations that the pandemic affects young adults with relative sparing of older age groups [19, 35]. A recent study found that preexisting antibodies may protect against pandemic influenza infection [19], whereas higher baseline titers may also independently reduce the likelihood of infection and consequently seroconversion [20, 37, 38]. Additional studies are needed to determine the effectiveness of baseline antibodies in reducing influenza infection.

Limitations of this study include the relatively few groups for comparison and the study's inability to separate the incremental impact of each individual intervention. These are intrinsic issues with observational cohort studies, even preplanned ones like ours, and additional studies should aim to look at other interventions, whether in combination or individually, where opportunities exist. We did not monitor participants after the epidemic to determine if cumulative case numbers trended toward parity over time for the different groups. However, national and military surveillance data showed that postepidemic ILI rates and percentage of ILI positivity for 2009 influenza A(H1N1) were low, and no major pandemic influenza outbreaks were detected in the military.

The measures adopted by the Singapore military were simple to implement rapidly, and the data reported here suggest that these public health measures—in particular, enhanced surveillance with isolation and social segregation—are likely to be effective in limiting influenza transmission and reducing the high attack rates during an epidemic in closed environments. These should be considered in preparation for future epidemics and pandemics, as well as in developing countries where pandemic vaccine coverage has not reached sufficient levels to prevent future outbreaks.

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Chapter Thirteen

Discussion and Conclusions

Although there have been many studies on different aspects of influenza over the years, there are still many areas that we do not understand about this important global threat. The 10 publications described in this thesis help to address some of these gaps and provide a substantial contribution to the existing scientific knowledge on influenza and its prevention. The findings from these studies are important for public health decision makers in this post-pandemic period during the revision of pandemic preparedness and response plans, and also to reduce the spread and impact of future seasonal influenza epidemics and outbreaks. This final chapter revisits the different concepts discussed in the previous chapters to identify lessons learned and their importance to policy making, suggest possible strategies for future implementation, and raises outstanding questions that need to be addressed in future studies. It also presents in the relevant context the limitations of these studies and the need for further studies to address some of these issues. This will enable researchers and policy makers to use the evidence base created by this thesis for planning in their local context.

Influenza in the Tropics

It is well known that influenza spreads globally with substantial morbidity and mortality. However, the spread and impact of influenza in the tropics has not been extensively studied. As shown in the third chapter and previous studies that I have published, tropical regions such as Singapore are not spared from the impact of influenza. In addition, many of the seasonal epidemics and pandemics that circulate globally also affect Singapore with regularity. However, the reasons for the unique timing of spread in terms of the main epidemic months remain unknown and are important to understand the spread of influenza in the tropics. One hypothesis for the

different patterns in the tropics relate to the timing of the global circulation of influenza viruses. Influenza viruses continually circulate in the tropics, especially in East and South-East Asia, resulting in overlapping epidemics which may seed the epidemics in temperate regions in both the northern and southern hemispheres (1). Other studies have shown that influenza viruses transmit better in animal models in conditions of lower temperature and lower humidity, while high humidity reduces viral survival (2,3). This may explain the high transmission during winter seasons in temperate countries, but does not fully explain the year round transmission with similar overall impact in the tropics without seasons and where high humidity abounds. Another hypothesis is that the transmission of influenza in temperate regions may be mostly by aerosol spread, while transmission in the tropics may be mostly by contact spread (4). As the evidence for influenza transmission in the tropics is lacking, more studies in this region on the circulation of influenza viruses is important. This may unlock the secrets to the global circulation of influenza viruses, and allow for international measures to be developed to reduce spread, especially during pandemics. These epidemiological studies should not only be focused on a few large developed cities in the tropical and sub-tropical regions where good data is available across time, but should also look collect data from less developed cities and rural areas to paint a more complete picture. Where data is unavailable, new surveillance studies should be set up to start data collection and lay the foundation for future studies.

The high baseline circulation of influenza throughout the year in the tropics with irregular timing of epidemic waves may also reduce the awareness of the population to the threat of influenza. This is unlike in temperate countries where influenza results in a reasonably consistent large epidemic wave during the winter months, prompting

measures such as vaccination to be taken by the local population. At this moment, there are few studies exploring the link between the patterns of influenza spread to the knowledge, attitudes and practices of the local populations. This will influence health education programs, public awareness and actions such as seasonal vaccination, and this should also be an area of future study. These studies will be crucial for those making policies to raise awareness of, and to advocate for, the use of preventive measures such as vaccination and other non-pharmaceutical measures.

Importance of Surveillance

Good surveillance systems, including laboratory capacity, are needed to answer the key questions on the spread and impact of influenza in the tropics and other regions, to examine the constant transmission of influenza in the tropics, and to determine the impact on the local population. Surveillance systems have to be customized for the local population, and be used on a routine basis to ensure that they are well managed and fully operational during epidemics and pandemics when they are most urgently required.

Surveillance is also key to the successful response to any epidemic or pandemic. As many of the studies in this thesis have shown, good preparedness and response planning is important to reduce the overall impact of influenza epidemics. The overall impact, which is influenced by the rate and extent of spread (including sub-populations at higher risk of infection), and the clinical severity of the disease, will determine the scope of the response. This is important as the response to any public health event must be proportional to the impact of the event. A disproportionate response is undesirable – for example, an over-zealous response compared to the

impact will result in resource wastage and indirect losses due to the response measures which may be more severe than the event itself. Examples of the latter include closure of borders which can affect travel and trade and negatively impact on the economy; while closure of schools for prolonged periods will result in economic costs and social issues. Conversely, an inadequate response will result in ineffective measures which will waste precious resources while not adequately reducing the impact of the event. As historical epidemics and pandemics (and previous trends) do not fully predict the outcome of future events (5), good surveillance systems are therefore needed to enable early epidemic detection for prompt measures to be taken, and also to determine key parameters such as the rate and extent of spread and the disease severity so that the response can be proportional to the impact.

Surveillance, especially laboratory-based surveillance of seasonal influenza, is also important to allow for a better understanding of the seasonal burden of disease and patterns of transmission, such that comparisons of impact can be made during epidemics and pandemics for proportional responses to be instituted (6). Surveillance must therefore be part of a routine system to ensure that capacities and capabilities are present to answer these critical questions. As local settings are unique, it is essential that surveillance strategies are tailored to the local situation, and this requires more local research to be performed.

However, influenza poses substantial and unique challenges for surveillance. As shown in Chapter Six, while it is possible to use various clinical symptom complexes to better identify influenza cases from non-influenza cases, it is inherently difficult to completely differentiate between them using clinical presentation alone. Where

possible, the use of clinical syndromes or symptom complexes should be supplemented by laboratory testing. The results from Chapter Four are similarly important for policy and decision makers, as they show that the estimation of infection rates using relatively simple ILI surveillance in primary healthcare settings, together with laboratory testing, can yield estimates that are comparable to more complex serological tests. The estimated numbers of laboratory tests that are needed during an epidemic to determine the epidemic curve and infection rates are also relatively small compared to the population at risk. This suggests that lower resourced countries and local areas can obtain samples from sentinel sites and send these samples to regional reference laboratories for testing, or to develop their own laboratory capacity as part of the national core capacity building to meet the requirements of the WHO's International Health Regulations. As the surveillance system that is most appropriate for each setting will vary according to the population distribution and interactions, additional feasibility studies should be performed in the local setting to determine the optimal surveillance system. This will need to take into account the healthcare system and available resources, and be tested and improved upon during local epidemics.

Throughout the 2009 pandemic, simple ILI and laboratory based surveillance systems were able to provide data on the development of epidemics early enough so that measures could be taken to reduce their spread, and to monitor the general shape of the epidemic curve. However, as the study in Chapter Four has shown, more data is required to accurately determine the overall infection rates. Using only unadjusted ILI numbers or laboratory-confirmed cases alone will result in gross underestimates in the infection rates, and may consequently result in overestimation of severity estimates

such as hospitalization and mortality rates. This occurred in the early data from Mexico during the 2009 pandemic which suggested high severity estimates, and prompted global fear that the pandemic would be much more severe than it actually was (7). In this context, it is necessary to have good data on the proportion of milder influenza cases and the health seeking behavior of clinical influenza cases among other variables, and this may be difficult to come by during the early phases of a pandemic. Additional studies will therefore have to be promptly performed during a pandemic to provide these data, and since it is often difficult to design and execute a study after the pandemic's onset, these study designs and resources have to be in place before the next pandemic.

Serological studies are a useful tool to provide good estimates of infection rates, to provide estimates of parameters such as the proportion of clinical to subclinical cases, and together with surveys to determine health seeking behavior of these cases. As shown in Chapter Five, the serological studies in different cohorts in Singapore allowed us to determine the extent of possible infection in the general community which showed that the majority of the population remained susceptible to infection, prompting pandemic influenza vaccination programs thereafter. It also showed the relative extent of possible infection in different sub-populations, and the predictors of infection in these populations. Where resources allow, serological studies should be considered as part of the surveillance system and study designs put in place a priori to enable prompt activation of data collection during the emergence of a novel influenza virus. These studies should also be done during seasonal epidemics to increase the understanding of infection rates and influenza transmission, and to show the effectiveness of interventions. However, serological studies have their limitations

which include the time lag to detection with current laboratory technology, the resource intensive nature from the laboratory testing, and with cohort studies where unique blood collections have to be performed.

For public health planners, it is therefore important to consider all possible surveillance solutions and adopt the best mix given the available local resources. Countries with sufficient resources should consider having different surveillance systems for cross referencing, and to maximize the advantages of each method while covering for their individual deficiencies.

Selection of Sentinel Sites

Sampling from sentinel sites is important for any surveillance system because it is not always possible to perform a comprehensive survey in the community or to sample from all possible sites due to the large sample sizes required. Sentinel sites therefore provide a reflection of the population being studied. In the selection of sentinel sites, it is important that these are chosen to enable early detection of epidemics, representativeness, and ease of data collection. In this aspect, as shown by the studies in this thesis, semi-closed communities that are closely linked with the general community and are at high risk for transmission are possible sentinel sites. This is because they often amplify any disease that is circulating in the community, and are more easily detected because of the intrinsic monitoring systems in place. For example, the military in the Singapore setting is similar to a boarding school where young adults gather during the week and return to the community during the weekends, often visiting community locations which provide a good conduit for disease transmission. These include malls, cinemas, nightclubs, religious and social

events, and other similar mass gatherings. It is not surprising that some of the first 2009 H1N1 outbreaks in Singapore occurred in the military setting, and some of these were linked to an outbreak in a popular nightclub.

Other early outbreaks in Singapore occurred in a church camp and school - similar semi-closed settings. In addition, the military provides free healthcare to the camp population (although individuals can also visit external healthcare facilities when they are outside the camp), and this ensures a relatively captive population where the majority of cases in an outbreak will visit the same facility and be easily noticed by the healthcare workers. This is in contrast to other mass gathering settings where individuals at the gathering disperse soon after and visit separate healthcare facilities when ill. Although it is certainly possible to piece together the epidemiologic puzzle to determine the presence and source of the outbreak, it may require more time and may only identify larger outbreaks.

However, in other countries, militaries may or may not be equally good sentinel surveillance sites as their structure and interactions with the general populations differ. This is especially true in larger countries where military camps may be located far from major population and travel centers. Schools, on the other hand, are quite similar in structure across countries – being located near population centers and having a population made up of children, youths, and young adults who frequently participate in mass gatherings. Schools are therefore a good setting for the surveillance of influenza and other diseases and sentinel surveillance sites can be set up in schools, and even within classes in each school. Monitoring of school students can be easily done by teachers, and in some schools onsite healthcare staff, as they

have a close and consistent relationship with the students. Such surveillance systems will have to be routine so they are functioning well ahead of any epidemic. Setting up surveillance systems in these settings during an epidemic itself is inherently difficult due to the approval processes, logistics, and training needed. I was part of a school surveillance study during the 2009 H1N1 epidemic in Singapore which was started at the onset of the epidemic itself, and can attest to the difficulties in setting up and obtaining good data from such a project. In comparison, the military surveillance studies shown in this thesis were much easier to execute due to the existing surveillance programs in the military which familiarizes staff to the different components and issues in performing surveillance. This ensures that even new systems such as collection of samples for serological testing, which is not routine, is easily performed because the surveillance principles are similar.

Measures for Preparedness and Response

While surveillance is necessary to detect the impending epidemic and determine the likely impact, it is important that measures are taken in response to the available information to reduce the impact – either on the spread of the virus, or the clinical severity of the infections.

Vaccines are one of the most effective measures to reduce both the spread and severity of infection. Seasonal vaccination has been shown to be effective in reducing infection rates, and have been encouraged for use in the general population (8) and with special emphasis in high-risk populations. However, as mentioned above, vaccination uptake rates in the tropics have been low, and this may be due to a lack of awareness and the behaviors of the local populations although there is a lack of

evidence in this area. Additional studies will therefore have to be done to understand the reasons behind the knowledge, attitudes, and behaviors of local populations to enable measures to be taken to increase the vaccination uptake rates.

Chapter Eight suggests that some cross protection exists between different influenza strains, and previous studies have suggested that the elderly may be less affected by the 2009 H1N1 pandemic strain due to pre-existing antibodies possibly obtained from pre-1957 H1N1 infections. However, the serological studies in this thesis in Chapters Five and Twelve found no significant protective relationship between previous seasonal influenza vaccination and serological conversion to the 2009 H1N1 pandemic strain. This is similar to a recent case-control study in Australia which showed no reduction in pandemic infection rates among those who had received the seasonal influenza vaccine (9). This Australian study also found no harm from seasonal influenza vaccine in increasing pandemic infection, which had been previously suggested as a possibility by a Canadian study (10). The latter may be due to the seasonal vaccine preventing natural seasonal influenza infection which provides non-specific temporal immunity, therefore leading to a higher infection rate among those immunized against previously circulating seasonal strains (11). There is currently a lack of studies showing how serological cross protection from infection or vaccination actually translates into protection against clinical infection, and a possible reduction in clinical severity even if full protection from infection is not achieved. More research is clearly needed in this area as this will influence policies on seasonal influenza vaccination for the general population, especially if vaccination reduces natural infection and immunity against different influenza strains.

Another research area that is promising is the development of universal influenza vaccines which are effective against a range of influenza subtypes and strains (12,13). This will address the major issues surrounding existing vaccines which are difficult to deploy during a pandemic – current stockpiling strategies involving candidate pre-pandemic vaccines for early deployment is a gamble on an unknown future pandemic subtype and strain, while the development of well-matched pandemic vaccines during the pandemic itself takes a long time (up to four to six months, excluding production of sufficient quantities to meet global demand). The availability of a universal vaccine would make the stockpiling of pre-pandemic vaccines more compelling and perhaps even cost effective for a wide range of countries.

In the absence of vaccines during a pandemic, anti-viral drugs and other non-pharmaceutical interventions are appropriate supplements that can be synergistic when used in combination. Many countries have been stockpiling anti-virals for treatment of influenza cases, pre-exposure prophylaxis of groups at high-risk of infection, and post-exposure prophylaxis of close contacts. However, there are few studies showing their effectiveness during epidemics or pandemics.

In Chapters Nine to Eleven, we have explored a novel use of these anti-virals and have shown conclusively that oseltamivir can be used for the protection of unique groups through ring prophylaxis to prevent the spread of influenza while maintaining their work functions. With this evidence, it is now possible to consider close monitoring of these essential personnel or populations at high-risk of infection, and to initiate ring prophylaxis only when initial outbreaks are detected. This is a shift from previous preparedness plans and should be considered in the future as a possible

effective and efficient use of national stockpiles to protect essential personnel without the need for prolonged pre-exposure prophylaxis, which expends large quantities of antivirals and are difficult to time well to coincide with the local epidemic (14). Such a strategy will be applicable to groups where it is necessary to maintain their work functions, where there are high-levels of group interaction, and where it is possible to monitor for initial outbreaks – examples of such settings would include hospitals, militaries and civil defense, and essential public services. Stockpiling of anti-viral drugs is costly even for well-resourced countries and are not likely to be cost-effective in the longer term for lower-resourced countries where two-thirds of the world's population reside (15). It is therefore important to consider different ways of maximizing the use of these drugs. Additional health economic studies are needed to determine the cost effectiveness of ring prophylaxis as a strategy to reduce the overall impact of the disease and reducing the need for treatment doses of the anti-viral drugs since less people are infected, against the quantities of the drug needed for the intervention.

For these pharmaceutical and other non-pharmaceutical interventions, one of the biggest challenges is in selecting the most appropriate set of measures at the global level that are proportional to the impact of the pandemic, and at the local level for the respective epidemics. This not only involves determining the impact, which poses its own challenges as mentioned previously, but also in the effectiveness and timing of the public health measures. There is a lack of field studies to show the effectiveness of individual or combinations of public health measures in real life. Such research is difficult because it is often not possible to perform randomized trials during an epidemic, and observational studies have to be done to determine the effectiveness of

prevailing measures in whatever setting is available. This necessitates the setting up of large scale research studies in different settings during various epidemics and pandemics to explore the large range of possible combinations of measures in different unique settings.

Given the demographic, economic, socio-cultural, political, and many other differences between settings, measures that are effective and feasible in one setting may not accrue the same level of effectiveness or feasibility in another setting. For example, the acceptance of (and compliance to) the measures by the local population is equally as important as there are many social, cultural, and economic reasons why different measures may be more or less acceptable in the local setting. These also have to be determined through behavioral research. In addition, access to health education, risk communication, and access to health care is important. Surge capacities may have to be considered in implementing many of these strategies. Given limited resources, it is also essential that the set of public health measures maximizes the use of available resources to ensure the best possible outcome. Health services research such as cost-effectiveness studies and resource optimization studies need to be performed in the local setting.

As there is a current lack of epidemiological and field data, it is also important to consider mathematical modeling studies to provide some evidence on the effectiveness of various strategies for policy making in the absence of definite studies. As mentioned in Chapter Seven, mathematical models can provide suggestions for decision makers to consider, while field studies such as those shown in Chapters Eight to Twelve are essential both to prove the effectiveness of the measures suggested by

the models, and also to provide additional data for future models. While there are many proponents and opponents of mathematical modeling, similar to many other situations, there is always potential merit in any activity but they must be used in moderation and in the correct context. Decision making using only mathematical models may be dangerous, especially if the models use assumptions obtained from other settings and when policy makers attempt to use these models to predict the future. Similarly, using only the limited epidemiological field studies available may result in similar problems if policy makers attempt to extrapolate these findings out of context. It is therefore essential to base critical decisions on all available information and tools, which often include both mathematical models and epidemiological field studies, and to analyze the context and assumptions under which they were performed. It is also essential to encourage additional research to validate these decisions where the opportunity arises, and this requires pre-planning and long term commitment. The later chapters of this thesis provide some epidemiological evidence to back up the findings of these models, as well as generating a new hypothesis for future studies.

Study Limitations

There are two key limitations for the overall thesis in addition to the limitations mentioned in the individual studies. One important limitations is the fact that many of the studies were performed in the military setting which is made up of young adults, mostly male, and mostly fit individuals (although conscription includes almost all fitness and medical conditions and provides appropriate job scopes to match the individual). In addition, the military is a semi-closed environment which has its own unique social mixing characteristics which will affect the spread of disease. While the

results of this study are relevant to similar groups, including other semi-closed environments, further studies are needed in the general population and specific sub-populations of interest such as schools, healthcare facilities, and among high-risk individuals to ensure that these findings are also applicable in those settings. The collection of studies in this thesis provides a good basis for hypothesis generation.

Another limitation to consider is the use of Singapore as the focus of the studies. Singapore is a unique city-state at the confluence of global travel, and a melting pot of different cultures and populations. As such, Singapore is able to provide an overall representation of the diversity of the tropics in a relatively small environment which is easy to study. At the same time, it is important to note that Singapore is an urban and well-resourced country and while it lends itself well to the availability of data for these studies, the same may not be available in other countries in the region or globally. In addition, social and behavioral differences will exist between countries, and socio-economic groups. It is therefore important to consider the findings of these studies as evidence on the importance of research on influenza and other diseases in the tropics, and to place additional emphasis on performing additional studies in the tropical regions for local relevance.

Conclusions

This thesis has contributed additional evidence on the spread of influenza in the tropics and the management of influenza outbreaks. It has answered some critical questions on the impact of influenza in the tropics, and proven the hypotheses on the effectiveness of various interventions such as anti-viral prophylaxis, and public health measures during influenza outbreaks. It has also generated additional questions and

hypotheses for future studies such as the possible cross protection of influenza vaccines, and the need for better clinical and laboratory diagnosis for clinical management and surveillance of influenza. The results of these studies should, in the near future, be validated in other sub-populations and in other settings to show their accuracy over a wider range of scenarios.

In addition, there are many similarities between influenza and other respiratory viruses, and influenza can be used as a good model for the management of outbreaks in the event of the emergence of yet unknown respiratory viruses. The SARS outbreak showed that these events can occur without warning, and there is the likelihood of another occurring in the future. Good preparedness and planning is therefore essential if we are to withstand the impact of future outbreaks and pandemics. Policy makers must therefore rely on the best available evidence and work together with researchers to ensure that evidence-based policies are made for the benefit of society.

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